METAL ORGANIC COMPLEXING IN SOIL SYSTEMS

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ABSTRACT

The work described in this thesis is concerned with properties of soil derived fulvic acids.

A method for isolating soil fulvic acids is described. The method makes use of the metal complexing agent pyrophosphate (pH 3) as the extractant. The hydrophobic XAD-7 resin was used for the selective separation of the non-ionic fulvic acids from the ionic extractant at low pH. Fulvic acids obtained after a single XAD-7 treatment had acceptably low ash contents (< 0.6%).

Chemical and physical properties of these fulvic acids were compared with those of fulvic acids that had been subjected to high and low pH conditions (conditions normally encountered during traditional methods of extraction). The resultant changes in molecular weight (gel exclusion chromatrography), equivalent weight and protonation constants (potentiometric titrations) and in metal binding properties (ion selective electrode potentiometry, fluorescence spectroscopy and anodic stripping voltammetry) are discussed.

The acid-base titration curves for fulvic acid have been interpreted in terms of fulvic acid having four structurally independent carboxyl groups, having mean pK_n values pK_1' .... pK_4'. The results for six fulvic acids were in the range log K_1' 7.2 - 6.5 ($\underline{c}$ 8% of total carboxyl acidity), log K_2' 5.9 - 5.3 ($\underline{c}$ 11%), log K_3' 5.0 - 4.1 ($\underline{c}$ 20%) and log K_4' 3.2 - 2.4 ($\underline{c}$ 60%). These residues indicate a low percentage (not more than 8%) of polyprotic acid residues (e.g. 1,2,3-tricarboxylic acid); these residues

may be important complexing sites.

A comparative study of fulvic acid complexing, both between fulvic acids and with model ligands, is reported. Ion selective electrode potentiometry, fluorescence spectroscopy and anodic stripping voltammetry were used to study copper(II) and lead(II) complexing. A variety of binding sites with different affinities for metal ions was indicated. The stronger binding sites are involved in binding at low metal concentration followed by weaker binding sites at higher concentrations of metal.

Copper(II)-fulvic acid binding curves (i.e. % free metal vs. pH) are similar to those for citrate, malonate and aspartate, and were modelled numerically by mixtures of these competing ligands.

The citrate ion was established as a useful model for describing the complexing reactions of fulvic acid. A detailed study has been made of the equilibrium reactions of citrate with protons and aluminium(III). The protonation constants for citrate are log K_1 5.90, log K_2 4.35 and log K_3 2.91. The model best describing the Al(III)-citrate equilibria was found to be $AlHL^{+}$ (log β 11.02), AlL (8.35), $Al(HL)L^{2-}$ (17.36), AlL_2^{3-} (13.40), $AlL_2H_{-1}^{4-}$ (pK AlL_2^{3-}, 6.07) and $Al(LH_{-1})_2^{5-}$ (pK $AlL_2H_{-1}^{4-}$, 7.09).

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Soil organic matter can be divided into two classes: non-humic (consisting of compounds belonging to recognised classes of organic compounds, e.g. amino acids, lipids, carbohydrates) and humic substances (high molecular weight, dark coloured compounds formed by secondary reactions). The latter class can be further divided into humic acids, fulvic acids and humin. Humic substances do not correspond to any unique chemical entity and accordingly cannot be defined precisely in structural terms. Consequently, they are defined operationally in terms of their solubility. Humin is the fraction that is not soluble in water at any pH. Humic acids are soluble in alkaline solutions, but precipitate on acidification. The precise pH of acidification is variable, but is commonly pH 2. Fulvic acids are the fraction of humic material that is soluble at all pH values.

This work studies the fulvic acid fraction of soil organic matter; its extraction from soils, its composition and structural features, and its ability to complex with metal ions from soils.

The soil is a dynamic system. It changes in response to any change in the soil forming factors - parent rock, topography, climate, vegetation/organisms and time. Soils are composed of a complex mixture of inorganic and organic components. These components may be in the liquid phase (e.g. soil moisture) or gaseous phase (e.g. CO_2, O_2, N_2), but are predominantly in the solid phase. The inorganic component consists mainly of rock fragments in the form of sand and silt,

and clays. These constituents vary in size and chemical composition, and hence give rise to texture in a soil. The organic component, consisting of humus, living and decaying vegetation, and organisms, also gives rise to soil texture.

The variation in soil composition allows soils to be classified. Soils may be described by their horizontal features (horizons) and by their vertical features (profiles). A soil may contain any of the following horizons in its profile, as described in sequence from the surface to the parent rock below (Figure 1.1) :

> The upper L, F, H horizon is an accummulation of organic Litter, Fermentation or Humified material over mineral soil.
>
> The A-horizon is an inorganic/organic rich horizon which may be leached producing an eluvial E-horizon.
>
> The B-horizon is an illuvial or accummulation horizon rich in sesquioxides and organic matter.
>
> The C-horizon is the unconsolidated, partially weathered parent rock. It rests on unaltered parent material (R).

Horizons may be divided into subhorizons, designated *s* (showing accummulation of sesquioxides Fe_2O_3, Al_2O_3), *h* (having excessive humus) or *g* (showing colour patterns representing periodic oxidation and reduction).

On a global scale, soil organic matter is important because it serves as a major reservoir of organic carbon.

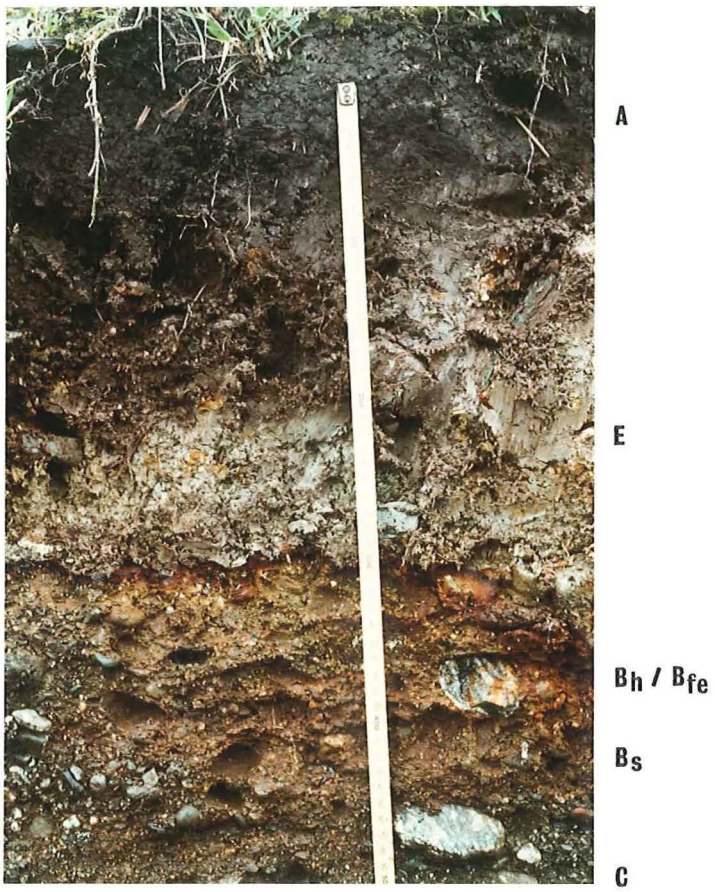


Figure 1.1 Profile of podzolic soil showing A-horizon, E-horizon, B-horizon (B_h, B_{fe} and B_s subhorizons) and C-horizon.

On a more local scale, organic matter affects the physical, chemical and biological properties of the soil, and consequently affects plant growth. Organic matter promotes good soil structure, improving aeration and retention of soil moisture, and resisting soil erosion. It provides a source of nitrogen, sulphur and phosphorous for plant growth, and affects the availability of many micronutrient cations through its exchange capacity. The action of herbicides and pesticides is influenced by the presence of organic matter. It may concentrate these compounds, influencing their biodegradability. Heavy metals, often toxic to plants and microorganisms, may also be concentrated by the soil organic matter. The presence of humic substances may influence a soil profile by enhancing the addition or removal of soil constituents from one horizon to another.

1.1.1 *Formation of Humic Substances*

The origin of humic materials has been a popular topic for debate. In the early twentieth century, two main theories were considered - the lignin theory and the sugar-amine condensation theory. Maillard's (1961) concept of sugar-amine condensation to form humic substances involved formation of sugars from carbohydrates and of amino acids from proteins by microbial metabolism of plant material. The reducing sugars then condensed (non-enzymatically) with the amino acids to form brown nitrogenous polymers.

The lignin theory, in which plant derived lignin was considered the precursor of humic acids, became generally adopted. According to this theory, lignins are modified and decomposed microbially, and the residues become part of the soil humus. These residues, modified possibly by condensation with proteins (Waksman, 1932), form humic and

fulvic acids that are strongly influenced by the nature of the plant material. In this theory the higher molecular weight humin and humic acids represent the first stages of humification. Fulvic acids, and ultimately CO_2 and H_2O, are formed by microbial decomposition of the larger acids.

A third theory, and now a favoured present day concept (Stevenson, 1982), is the polyphenol theory. Phenolic aldehydes and acids released from lignin by microorganisms, or polyphenols synthesized by microorganisms from non-lignin carbon sources, are enzymatically converted to quinones. The quinones undergo self condensation or condense with amino acids to form large nitrogen containing polymers. The vast number of precursers and ways in which they could combine account for the heterogeneous nature of humic substances. Unlike the lignin theory, large molecules are built up from small starting materials. Thus fulvic acids would form initially, and through condensation and polymerization reactions form humic acids.

Whether or not fulvic acids are formed from humic acids, or vice versa, or whether the two types of acids are formed by different pathways has not yet been resolved. Humic substances *may* be formed by all of these methods, with the dominant method in any one soil being determined by the type of soil and the environmental conditions.

1.1.2 *Sources of Fulvic Acid*

Humic substances are widely distributed on the earth's surface. They are found in soils, lakes, rivers, sediments, peats and the sea.

Peats are organic rich soils and are potential sources of humic substances. However they also contain large proportions of unhumified material.

Lakes and rivers contain dissolved organic matter, often to an extent that the waters become significantly coloured. The source of the organic matter is most likely run-off from the surrounding soils. River and lake sediments accumulate humic substances, either as precipitates from the waters or by adsorption on clay surfaces.

Humic matter is found in all horizons of the soil profile, but is more obvious in zones of accumulation, generating dark coloured regions. Sites of accumulation are often associated with routes of water flow. For example, worm tunnels and root channels become coated with humus. The illuvial B-horizons of a soil, specifically the B_s and B_h subhorizons, are major accumulation sites, and are commonly used as source material for extraction of fulvic acid.

The close association of B_s and B_h subhorizons has lead to the implication that fulvic acids are involved in the degradation and movement of mineral matter in soils. One particular type of soil of current interest is the podzol soil. These soils are characterized by eluvial E-horizons low in sesquioxide coatings, and illuvial B-horizons rich in sesquioxide coatings (B_s) and organic matter (B_h). The organic matter may preferentially deposit in a layer above the B_s subhorizon. A debate continues concerning the mechanism of the podzolization process. The most widely held view at present involves the movement of iron and aluminium as soluble fulvate complexes. As the metal : organic ratio increases the complexes become insoluble and are precipitated, generating the soil profile described above. In contrast, an alternative mechanism involves movement of iron and aluminium as soluble iron-aluminium-silicate sols (Farmer, 1982). A two-stage process occurs. Firstly, allophane and associated iron oxides in the B_s horizon are deposited from a mixed

Al_2O_3-Fe_2O_3-SiO_2-H_2O sol. Secondly, the B_h horizon is formed by anionic fulvic and humic acids which have passed through the A-horizon and E-horizon, then precipitate on the positively charged sesquioxide-coated B_s horizon.

These opposing mechanisms proposed for podzol soil development highlight the need to understand the nature of metal - fulvate complexes.

1.2 COMPOSITION AND STRUCTURE OF FULVIC ACID

Fulvic acids are a heterogeneous mixture of organic components. They are defined as the fraction of humic material that is soluble in both acid and alkali.

1.2.1 *Elemental Analysis*

The predominant elements in fulvic acids are carbon (40 to 50%), oxygen (45 to 50%) and hydrogen (3 to 6%). Nitrogen and sulphur are present in lesser amounts, the nitrogen from 1 to 3% (Stevenson, 1982). Elemental analysis is of little value in functional group analysis or structural determination because if offers only total elemental composition.

1.2.2 *Functional Group Analysis*

(a) Chemical Methods

More specific chemical analyses are required to determine the distribution of the elements in functional groups. The acidity of various functional groups is made use of in these methods. However, because of the heterogeneous nature of the organic matter, many characteristics of these groups may overlap. For this reason considerable disagreement exists regarding the exact composition of humic substances.

The major oxygen containing functional groups reported

include carboxylic (57-75% of the total oxygen), alcoholic (11-16%) and phenolic (9-19%). Several methods for determining each of these groups are used, but the most common methods are as follows (Stevenson, 1982; Schnitzer and Khan, 1972):

1. Total Acidity - the barium hydroxide method.

   The sample is reacted with excess $Ba(OH)_2$ and an insoluble barium precipitate is formed with the conjugate base of the organic matter. The remaining $Ba(OH)_2$ is back titrated with standard acid.

2. Carboxyl Acidity - the calcium acetate method.

   Humic substances liberate acetic acid when reacted with calcium acetate. The liberated acetic acid is titrated with standard alkali.

3. Total Hydroxyl.

   This is determined either by methylation with dimethyl sulphate, where the resulting precipitate is analysed for $-OCH_3$, or by acetylation with acetic anhydride, where the resulting acetyl content is determined by titration.

4. Phenolic Hydroxyl - by difference.

   total acidity - carboxyl acidity.

5. Alcoholic Hydroxyl - by difference.

   total hydroxyl - phenolic hydroxyl.

Whereas almost 100% of the oxygen can be accounted for by functional group analysis, nitrogen is incompletely accounted for by recognisable components (amino acids, amino sugars and ammonia). Of these three classes, the amino acids represent the greatest proportion of nitrogen

(30-40%). The next largest identified class is ammonia (17-20%), with amino sugars accounting for 2 to 5% of the nitrogen. This leaves 50 to 65% of the total nitrogen unidentified (Schnitzer, 1985).

(b) Physical Methods

Non-destructive spectroscopic techniques are commonly used to characterize fulvic acid functional groups. However, because of the variety of components in these acids, many of the methods provide little more than a blunt tool for identifying general characteristics.

Ultraviolet and visible spectra of fulvic acids often show little more than a featureless curve that increases in intensity with decreasing wavelength. In the visible region of the spectrum, the ratio of absorbances at 465 nm and 665 nm (E_4/E_6) is used for comparison of samples. For humic acids this ratio is typically between 3 and 5, whereas for fulvic acids it ranges from 6 to 8.5. The E_4/E_6 ratio increases with increasing molecular weight and condensation, and may serve as an index of humification (Stevenson, 1982).

Infrared spectroscopy, in contrast to ultraviolet and visible spectroscopy, does yield spectra with a variety of bands. The frequencies of these bands are characteristic of various structural units. Dominant groups that have been identified include OH groups, aldehyde, ketone and carboxyl groups, amide linkages, aliphatic and aromatic carbons and protons, and polysaccharides (Stevenson, 1982).

Nuclear magnetic resonance spectroscopy, both ^{13}C and ^{1}H, has been applied to fulvic acid samples. The spectra often show broad and poorly resolved resonances resulting from the extreme molecular complexity of the samples. Data from nmr spectra indicate that fulvic acids are composed of

both aromatic and aliphatic carbon structures. They contain carbonyl, carboxyl and phenolic groups, as well as C–O linkages that could be in alcoholic, ester or ether groups (Wershaw, 1985).

1.2.3 *Structural Units*

To provide information on the structural units of humic substances, degradative methods have been used to produce simpler compounds whose chemical structures are fragments of the starting material. Some earlier methods were too mild to yield identifiable products, and some so drastic that only acetic acid, CO_2 and H_2O could be identified. In principle, the ideal method should be mild, but yield high amounts of degradative products of moderate molecular complexity, while minimizing the formation of artifacts (Stevenson, 1982). Oxidative methods, including alkaline permanganate, alkaline cupric oxide, alkaline nitrobenzene, nitric acid and H_2O_2, have been applied. More recently, methods have been used in which labile oxygen-containing functional groups have been protected by methylation prior to degradation. Reductive methods such as zinc dust distillation and sodium amalgam reduction have also been used.

Numerous identifiable compounds have been found in the degradation products of fulvic acids, including benzene carboxylic and hydroxycarboxylic acids, methoxy substituted carboxylic acids (e.g. syringic and vanillic acids and aldehydes), substituted phenolics (e.g. catechol, resorcinol) and aliphatic acids.

Methods of hydrolysis with water, acid and alkali have provided limited information. Neyroud and Schnitzer (1975) concluded that alkaline hydrolysis was suitable for

degrading phenolic components and for liberating adsorbed aliphatics and nitrogen containing compounds. However, it was ineffective in degrading aromatic C—C linkages. These linkages could be degraded by oxidation with alkaline permanganate to yield benzenecarboxylic acids. The products were identified by gas chromatography following methylation. Major products identified after alkaline permanganate oxidation of humic substances included benzencarboxylic acids (di-, tri- and tetra-), phenolic acids and aliphatic dicarboxylic acids.

Humic substances are generally considered to be predominantly aromatic and phenolic in character, largely on the basis of high yields of benzene and hydroxybenzene polycarboxylic acids obtained by oxidative procedures. There has been increased interest in the aromatic nature of humic substances since the advent of non-destructive solid state ^{13}C nmr spectroscopy.

Bayer et al. (1984) concluded from solution and solid state ^{13}C nmr spectra that humic substances from waste water were dominated by aliphatic structures, not aromatics. Only weak signals were observed in the arene region (120-160 ppm), and very intense signals were observed from the saturated aliphatic region (10-40 ppm). From intense signals at c 70 ppm, they suggested that the aliphatic ethers assumed a dominant part of the oxygen containing groups. Farmer and Pisaniello (1985) also favoured a minor aromatic composition for soil fulvic acids, claiming that the aromatics were probably artifacts or contaminants introduced during degradation, isolation or identification.

Proton nmr spectra have indicated a high proportion (>72%) of aliphatic components in marine humic and fulvic acids (Harvey et al., 1983). Marine fulvic acids have few

or no aromatic protons. Barton and Schnitzer (1963) also noted the absence of aromatic protons in spectra of methylated humic acids, and suggested that it may be caused by the aromatic nuclei of humic substances being fully substituted by atoms other than hydrogen, or relaxing effects of the spins of unpaired electrons interfering with the ^{1}H nmr measurements. The aromatic nature of humic substances is still a matter of debate.

1.2.4 *Molecular Weight*

Average molecular weights and molecular sizes of humic substances have been estimated by a variety of methods. Molecular weights are commonly determined by methods including ultracentrifugation, viscometry, vapor pressure osmometry and freezing point depression. However, these methods are not without their limitations and consequently there is little agreement on molecular weights of humic substances determined by the various methods. Because of the complex mixture of organic substances, methods usually determine 'average molecular weights'. These may be either 'number average' (weight/number of molecules present) or 'weight average' (Σ(weight x molecular weight)/total weight) values. 'Number average' molecular weights ranging from 500 to 2000 are typical for fulvic acids (Stevenson, 1982).

Molecular size techniques, including gel filtration, ultrafiltration and small-angle X-ray scattering are based on comparisons with model compounds of known molecular weight and composition. However, the compounds used for calibration may not be valid for humic substances because of the wide variation in composition, charge and shape of the humic material. Careful consideration of experimental conditions is required because the charge and shape of the molecules can

vary with pH and ionic strength, affecting their movement through gels and membranes.

Molecular size studies of humic acids, using low-angle X-ray scattering (Wershaw et al., 1977; Wershaw and Pinckney, 1977, 1978; Wershaw, 1986) has lead to a new model for the structure of humic acids, fulvic acids and humin. The model proposes a hierarchial structure where smaller units are linked together by weak interactions (hydrogen bonding, pi-bonding, hydrophobic interactions) to form aggregates. The resulting micelle or membrane type structures contain hydrophobic interiors and hydrophilic exteriors. The degree of aggregation in solution, and hence the apparent molecular size, is a function of the extent of hydrogen bonding. The aggregates can be destroyed by eliminating hydrogen bonding, either by raising the pH, or by replacing the acidic protons with methyl groups.

1.3 ACID-BASE PROPERTIES OF FULVIC ACIDS

Fulvic acids are weak, polymeric acids, consisting predominantly of carboxylic and phenolic functional groups. The degree of ionization of these groups has a profound effect on fulvic acid properties in aqueous systems. Fulvic acids are extremely soluble in natural waters. This can be attributed to the high proportion of carboxyl groups (see Section 1.2.2) which will be ionized at the pH of such aqueous systems. The ability of fulvic acid to complex metal ions is dependent on the ionic state of fulvic acid. Not only the overall ionic state, but also the proportion of various acidic functional groups will affect the extent of metal complexing. Therefore determination of the strengths (pK_a) and concentrations of the acidic groups may serve as an index to complexing capacity.

Traditionally, the carboxyl group content of humic substances has been determined by the calcium acetate exchange method and the phenolic groups calculated as the difference between the total acidity (as determined by the barium hydroxide method) and the carboxyl group concentration. The analysis of potentiometric titration curves can also yield useful information on the acidic nature of these acids.

1.3.1 *Interpretation of Titration Curves*

The shape of a fulvic acid titration curve is a gradual rise in pH with added base, showing buffering over a wide pH range. This is indicative of several groups with different acidities.

Probably the most obvious information available from an acid-base titration curve is the end point titre. This gives information on the number of titratable protons in a sample. The equivalent weight is defined as the weight of fulvic acid divided by the moles of titratable protons. The lower the equivalent weight the greater is the acidic content of the sample. Because of the complex nature of fulvic acid, sharp inflections indicating equivalence points are rarely observed. Thus, difficulty in determining total concentrations of acidic groups arises. Martin and Reeve (1958) arbitrarily chose pH 8.0 as the end point, and Posner (1966) used pH 7.0-7.6 where the rate of change of pH was at a maximum. A modification of the Gran function was used by Pommer and Breger (1960) to define humic acid titration end points.

Another difficulty often encountered during titrations of fulvic and humic acids is the time taken for equilibrium to be established following each increment of alkali. Discontinuous titrations, where the pH of aliquots of humic solutions containing increasing amounts of acid or alkali is

measured, have been used (Borggaard, 1974 ; Pommer and Breger, 1960). However, Borggaard also reported that humic titrations should be carried out as quickly as normal titrations to prevent atmospheric oxidation of acidic functional groups. Others (Khan, 1969; Posner, 1966) have used automatic titrators to perform slow titrations, allowing sufficient time for equilibrium to be established.

Conductivity titrations also yield end points. During a conductivity titration the observed resistivity (reciprocal of conductivity) increases as the protons are replaced by cations (K^+ or Na^+, say). Protons have a much higher specific conductivity than the cations K^+ or Na^+. Beyond the end point, hydroxide ions, also of high conductance, dominate and result in a decrease in the observed resistivity. For an acid like fulvic acid where many different acidic groups are likely to be present, there may be more than one conductivity end point.

1.3.2 *Analysis of pH Titration Data*

To account for the complexity of the acidic nature of fulvic acids, a variety of models have been applied to acid-base titration data. These models are usually based on one of the following general descriptions :

(i) the discrete models that postulate two or three distinct binding sites with unique protonation constants,

or (ii) the continuous distribution models that assume humic substances contain a continuous distribution of acidic functional groups.

Probably the most common approach used is the extended Henderson-Hasselbach equation. It considers that all groups

in the polyelectrolyte are chemically equivalent, and are described by the equation

$$pK_m = pH - n\log(\alpha/(1-\alpha))$$

where α is the degree of dissociation, and pK_m is the apparent pK at $\alpha = 0.5$.

A variation of this function is that used for the electrostatic polyelectrolyte model,

$$pK_{APP} = pK_{INT} + 0.868\ m\alpha W$$

This assumes a continuous increase in apparent dissociation constant (pK_{APP}) with increasing degree of dissociation (α). The increase is caused by a build-up of charge on the polymer during proton dissociation, retarding further dissociation from identical carboxyl groups. The lowest value obtained (pK_{INT}) corresponds to a state of zero charge on the molecule.

Young et al. (1981) commented that the electrostatic model of identical group ionization may only be applicable to high molecular weight acids (such as humic acids) with low densities of isolated carboxyl groups. The lower molecular weight fulvic acids have a greater range of carboxyl group densities which may render the groups chemically dissimilar.

Purely electrostatic explanations of proton binding in humic substances are not adequate. Several authors realised that a variety of protonation constants must exist in humic substances. In a complex mixture of ligands (such as fulvic acid) some ligands will have a stronger affinity for protons than others. It is well known that different classes of functional groups (such as carboxylic and phenolic) will have quite different protonation constants. It is also well

documented that within a class of ligands slight structural variations will be reflected by variations in protonation constants. Therefore, it is reasonable to assume that the continuous distribution of stuctures in fulvic acid would be reflected by a continuous distribution of protonation constants.

Gamble (1970, 1972) and Burch et al. (1978) considered fulvic acid as an irregular polymer with a number of chemically non-identical acidic functional groups whose respective dissociation 'constants' were a function of the overall degree of ionization of the polymer. Gamble reported the existence of at least two types of acidic functional groups in fulvic acid. The most acidic of these was thought to be carboxyl groups which were ortho- to phenolic -OH groups; support being given by comparison with dissociation constants for hydroxybenzoic acids. Evidence for these carboxyl groups was based on conductivity titrations in which conductance minima were observed at lower titre values than the end point of the corresponding potentiometric titration.

Arp (1983) examined acid-base titration data of humic substances using Nernst-like and double-layer models which simulate proton-affecting surface potentials. Each distinct section of a titration curve represented a collection of functional groups with a range of overlapping K values, and was characterized by an average K value.

A model, assuming fulvic acid can be treated as a limited number of monoprotic acids, has been applied by Paxeus and Wedborg (1985). Electrostatic effects were ignored. The protonation constants and associated concentrations were estimated by minimizing a function of hydrogen ion concentration, $[f(K_i, \text{concentration}) - f(\text{pH}, \text{titre})]$, over all titration points. The 'correct' model was based on the statistical improvement obtained when the number of parameters was increased from three

to fifteen. The titration of fulvic acid in the range pH 2.5-10.5 was best fitted as six monoprotic acids. The three most acidic groups were carboxylic.

Erble and Feuerstein (1979) reported a 'discrete pK spectrum' for humic acid. Using fixed pK values at intervals of $\underline{c}$ 0.5 pK units, the pK vs. concentration spectrum showed six concentration maxima. In contrast to this 'discrete pK spectrum' technique, Leuenberger and Schindler (1986) generated a 'continuous pK spectrum' for fulvic acids. The approach defines r average concentrations for r intervals of pK. Each interval contains an infinite number of pK values. The greater the resolution (r) the closer the calculated concentrations approximate to the actual concentrations.

As a ligand becomes increasingly complex, titration curves become more and more featureless. Humic substances clearly show their acid-base complexity with featureless titration curves and poorly defined end points. Almost any function with several variable parameters will fit experimental data. This is well illustrated by the variety of chemical models that have been applied. However, not only does a model have to fit the experimental data, but it must also yield chemically sensible parameters that reflect the complex acidic nature of fulvic acid.

1.4 CHEMISTRY OF METAL-FULVIC ACID COMPLEXING

The importance of understanding metal-fulvate interactions was alluded to in Section (1.1.2), in relation to soil forming processes. Metal-fulvate interactions are also important in the transport and bioavailability of metals in aquatic systems. Tuschall and Brezonik (1983) point out four important aspects of metal binding by humic substances that determine the availability of uncomplexed metal ions:

1. The capacity of the organic matter for binding metal ions.
2. The stability of the complexes.
3. The lability of the complexes.
4. The rates of formation of the complexes.

There has been considerable work in determining the capacities of humic substances for binding a variety of metal ions. Study of the stabilities of these complexes has also received much attention, but determining stability constants for these large, complex organic molecules is difficult. This is discussed in detail in Section (1.4.2). Over more recent years some effort has been directed toward the study of labilities of these complexes.

1.4.1 *Metal Binding Sites in Fulvic Acids*

As discussed in Section (1.2), fulvic acids contain both aliphatic and aromatic units. Functional group analyses indicate a large proportion of carboxylic and phenolic groups, and it is the presence of these acidic oxygen containing groups that is thought largely responsible for the complexing ability of fulvic acids. These functional groups have a known propensity for binding metal ions. Other functional groups such as carbonyl, amino and imino groups may, to a lesser extent, be involved in complexing.

Salicylate moieties (having a carboxylic group ortho to a phenolic group) have been implicated as important binding sites in fulvic acids (Schnitzer and Skinner, 1965). These binding sites were determined by selectively blocking combinations of alcholic, phenolic and carboxylic groups, and measuring the metal retention capacity of the organic matter. Gamble et al. (1970) and Takamatsu and Yoshida (1978) have

suggested adjacent carboxylic groups, either on aromatic rings (phthalate type) or on aliphatic chains, as a second important binding site. Buffle et al. (1980) recognised the need for the presence of two or more types of binding sites the observation that the strength of binding increases as the metal:ligand ratio decreases.

If a ligand (e.g. fulvic acid) has binding properties similar to known model ligands, then it is tempting to postulate similar structural properties. Cressey et al. (1983) deduced from comparative studies, using ion selective electrode potentiometry, that in weakly acidic solution copper and cadmium complexing by fulvic acid was best modelled by aliphatic α-hydroxy carboxylic acids such as malic and citric acids. For neither metal did they find the salicylate unit a satisfactory model.

1.4.2 *Critical Comments on Methods for Determining Stability Constants*

The quantitative measure of the affinity of a metal ion for a ligand is the stability constant. However, numerous problems are encountered in determining stability constants for metal-fulvic acid complexes. For a macromolecule like fulvic acid, where several types of binding sites may be present, the stability of a complex will be dependent on the reactivity of binding sites, i.e. the strongest binding sites will be the first to complex, followed by the weaker sites at higher loadings of metal ions. The preferred binding site may change with pH.

The stability constant expression is defined in terms of concentrations of the various equilibrium species (free metal, complexed metal, free ligand), usually in units of moles per litre. In the case of fulvic acids, it is not possible to express concentrations in these units. The concentration

of the uncomplexed ligand is commonly expressed as the reactive site concentration, i.e. concentration of carboxylic and phenolic groups. This may be determined by potentiometric titration. A measure of the amount of complexed ligand or metal is also required, and a variety of methods have been applied. These include ultrafiltration, dialysis, ion selective electrode potentiometry (ISE), hydrogen ion potentiometry, anodic stripping voltammetry (ASV) and fluorimetry. The validity of results obtained from any method of speciation analysis is dependent on recognising the limitations of the method. A discussion follows outlining the limitations of various methods when applied to the study of metal-fulvic acid complexing.

Separation techniques such as *gel chromatography, ultrafiltration* and *dialysis* are so called because they physically separate free metal from complexed metal by selective gel or membrane retention. The free metal fraction can then be analysed by standard techniques. Three major problems arise when using gels or membranes:

1. Adsorption of organic matter on the gel or membrane, altering the retention characteristics.
2. Disturbance of equilibria by removal of one component from the system.
3. Loss of small complexes through the membranes.

Membranes are usually calibrated by use of proteins of known molecular weight. However, for fulvic acids the molecular weight may not be directly related to the size of the molecule due to changes in conformation with changes in ionic strength and pH. Thus, molecules that would be predicted to be retained on the basis of molecular weight may pass through a membrane if they are compact in size.

The *ion selective electrode* method detects free metal ions, but is limited to those metals for which specific electrodes have been developed, e.g., Cu, Cd, Pb. Calibration of the electrodes is usually carried out in the absence of ligand; metal buffer solutions extend the calibration to lower concentrations ($< 10^{-6}$ M). However, adsorption of organic matter on the electrode surface is a problem and may result in a deviation in electrode response (Saar and Weber, 1982). Electrode calibration should be carried out in the presence of ligand. Adsorption may also result in drifting potentials during a titration. One major limitation of this method is the need to study systems with metal concentrations much higher than would be found in nature.

Hydrogen ion potentiometry has been used to determine the stability constants for copper - fulvic acid complexes (Young et al., 1982) and aluminium - fulvic acid complexes (Young and Bache, 1985). In this method, the magnitude of the pH drop on addition of metal ions to fulvic acid solutions is taken as a measure of complex formation.

$$M + n\ HL \quad \Leftrightarrow \quad ML_n + n\ H^+ \quad .$$

The stability constants were calculated using the knowledge of variation of pK_{APP} for the ligand with polymer charge. The equilibrium concentration of free ligand was calculated at any given datum point by an iterative procedure, solving the equation

$$[L^-] = [LH]K_{APP}/\alpha \quad .$$

The concentration of protonated carboxyl groups was calculated directly from

$$[LH] = \text{total ligand} - [Na^+] - [H^+] + [OH^-] \quad .$$

The concentration of the complex ML_n was then calculated by solving the equation

$$\text{total ligand} = [L^-]+[HL]+\Sigma nML_n$$

and substituted into the stability constant expression. One invalid assumption used in this approach was that the concentration of deprotonated ligand was equal to the concentration of free binding sites. It was not recognised that it is possible for acidic groups on the ligand to deprotonate but not participate in metal complexing.

One of the most widely used methods for studying metal speciation in natural systems is *polarography*. Although other polarographic techniques have been used, ASV is commonly used because of its low detection limit (10^{-7} - 10^{-9} M). Despite its wide use, the ASV technique does have problems. One problem that has been common to several of the methods discussed already is that of adsorption. Organic matter is known to adsorb to the surface of the mercury drop (Florence, 1986a). The adsorbed layer of organic matter may hinder the diffusion of metal ions to the drop, either by forming non-labile complexes or by generating a physical barrier. Alternatively, it may also be responsible for concentrating metal ions in the vicinity of the drop, enhancing signals under appropriate conditions. Consequently adsorption is likely to lead to a nonlinear relationship between stripping current and deposition time. Stability constants have been determined on the basis of peak potential shifts, but adsorption of fulvic acid may also lead to shifts in peak potentials.

The measured current of an ASV polarogram results from two processes, the diffusion current caused by reduction of free metal ions and metal ions in labile complexes, and the

kinetic current which arises from the reduction of metal ions from complexes which have partially dissociated during the lifetime of the electrode process. Determination of stability constants requires the concentration of free metal ions only. Therefore the technique cannot be applied to systems with labile complexes, and the measured current has to be corrected for the kinetic current contribution. Another contributer to the observed current may be the direct reduction of a metal complex. The peak height (proportional to current) is not solely related to processes occuring during the plating step, but is also related to processes occuring during the stripping step. If an intermediate oxidation state can be stabilized (say, Cu^{+} by $C\ell^{-}$), then the observed peak height will be diminished. Broadening of waves may occur as a result of adsorption and from the formation of a variety of similar but not identical complexes during the oxidation process. Thus peak height may not be the best measure of current, and peak area should be considered. The peak area will remain the same provided the same number of electrons are involved, regardless of the form of the oxidized metal.

In contrast to all of the previous methods discussed, *fluorescence quenching* (FQ) measures the concentration of free ligand. Fulvic acid is known to contain fluorescent centres, and this fluorescence can be quenched by complexation to paramagnetic metal ions. Thus, the fluorescence intensity will decrease during a titration as the metal ions complex. Two criteria must be met before FQ can be used to study metal-ligand complexing. Firstly, uncomplexed metal ions must not quench fluorescence, and secondly FQ must be proportional to the amount of metal complexed. Of the structural components of fulvic acid, it is likely that only the phenolic units (e.g. salicylate) will fluoresce. However, if complexing

occurred through nonfluorescent sites, such as citrate or malonate units, then no FQ would be observed. Working with an excess of copper ions, Ryan and Weber (1982) observed substantial residual fluorescence after titration in the presence of ligand ($\underline{c}$ 20%). This residual intensity was attributed to either the fluorescence efficiency of the complex, or to the possibility that nonbinding fluorescent sites exist. Tuschall and Brezonik (1983) also observed incomplete FQ by copper and suggested that not all fluorescent sites were involved in complexing.

Many experimental factors can alter the results of speciation studies. The method of analysis and its pitfalls are not the only factors. Another important factor is the source of the fulvic acid and the method of extraction used. Not only may the organic matter be altered during extraction, but the extraction may lead to a product with a high metal content. These metal ions may already occupy binding sites, forcing the added metal to complex with weaker binding sites. The degree of metal loading of the ligand (i.e. metal:ligand ratio) can affect the types of complexing sites involved. Data manipulation, or choice of which equilibrium model to apply, is just as important as data collection in generating meaningful stability constants.

Models which have been applied to fulvic acid - metal complexing include the following :

(i) The multi-site model, where it is assumed that binding takes place at a small number of distinct binding sites. Each site is defined by a stability constant and a concentration.

(ii) The multidentate model, where it is assumed that there is a single binding site, but a bidentate complex is possible.

(iii) The electrostatic model, where again a single binding site is assumed, but the stability constant is dependent on the net charge on the ligand.

(iv) The normal distribution model which assumes a continuum of binding sites with log K values distributed in a normal mode.

(v) The affinity spectrum model which also assumes a continuum of binding sites. However, the distribution is not required to be normal. A frequency distribution of log K values is obtained.

1.5 EXTRACTION PROCEDURES

In soils fulvic acids are present as soluble and insoluble complexes of metal ions, and as complexed or adsorbed acids on clay surfaces. To release the fulvic acids, one may extract with

(i) Hydroxide, by forming metal hydroxides.

$$OH^- + M\text{-}FA \rightarrow FA^- + A\ell\ (OH)_x\ ,\ Fe(OH)_x$$

(ii) acid, by protonating the organic matter.

$$nH^+ + M\text{-}FA \rightarrow H_nFA + M^{n+}$$

or (iii) a competing ligand, displacing the organic matter from sites of attachment on the clay-sesquioxide matrix.

1.5.1 *A Survey of Extraction Procedures*

The first attempts to isolate humic materials from soils used alkali, and today alkali remains the most widely used and effective extractant. Various modifications to the

alkali method have been made to increase the efficiency of the extraction and to decrease the extent of alteration of organic matter. Holtzclaw et al. (1976), Levesque and Schnitzer (1967) and Weber and Wilson (1975) all used 0.5 N NaOH for the extraction of humic materials from soils. Malcolm (1976) and Lowe (1975) used more dilute alkali (0.1 N NaOH), but this is reported to extract more inorganic material than higher alkali concentrations and hence leads to material with higher ash contents (Levesque and Schnitzer, 1967).

For a more complete extraction of soils with high polyvalent metal ion contents, leaching with alkali is often preceeded by a dilute acid treatment.

In general, the work-up procedure following extraction involves separation of the humic acids from the fulvic acids by acidification (pH $\leq$ 2) to effect precipitation of the humic acids. Purification of the fulvic acids is achieved either by dialysis, by ion exchange or by resin treatment.

Neutral salts of complexing agents have been used as extractants, but the yield of humic material is usually low. The complexing agent competes with the organic matter for the metal cations that bind the organic matter to the surface of clays, and thereby releases the organic matter. In a study of 80 extraction methods, pyrophosphate at pH 10 extracted more organic matter than alkali, orthophosphate, sodium fluoride or organic acids at the same pH (Bremner and Lees, 1949). The extraction of humic matter by pyrophosphate is increased with increasing pH above pH 8.6.

Pierce and Felbeck (1972) proposed a sequential extraction scheme that progressed from mild to more severe conditions. Of the three sequences studied, the following was found to be the most satisfactory, extracting 51-74% of the organic carbon: benzene-methanol, 0.1 M HCℓ; 0.1 M

$Na_4P_2O_7$; 6 M HCℓ at 90°C; $CHCℓ_3$-methanol, and finally 0.5 M NaOH.

Goh (1970) modified the method of Gascho and Stevenson to recover humic and fulvic acids with low ash contents. This sequential extraction involved dialysis (against 0.1 M HCℓ, then 0.3 M HF, then distilled water) as a pretreatment to remove most cations. The organic matter was extracted by dialysis against 0.2 M pyrophosphate at pH 7.0 and then against 0.03 M NaOH. Further dialysis and ion exchange was required to purify the organic matter. The fulvic acids, and any low molecular weight polymers passing through the dialysis tubing were recovered by adsopriton on Polyclar AT (polyvinyl Pyrrolidone), followed by desorption with 0.03 M NaOH.

Ion exchange resins have also been used for extraction of humic material. Levesque and Schnitzer (1967) used Dowex A-1 resin in sodium and acid forms, and compared the products with those obtained from NaOH extraction. The products were found to contain twice as much ash as those extracted with alkali. de Serra and Schnitzer (1972) compared the products of extraction with 0.5 M NaOH and sodium-Dowex A-1 resin. They too found high ash contents (> 40%) in the resin sample, but were able to lower the ash content to $\underline{c}$ 5% by purification with HCℓ-HF.

Martin and Reeve (1955, 1957a, 1957b) studied the use of the organic reagents acetylacetone and Cupferron, and found them capable of extracting considerable amounts of organic matter from podzolic B-horizons. Cupferron, 0.064 M, was the most efficient, removing 94% of the total carbon. However, the reagent was not entirely satisfactory because excess reagent was difficult to remove. Acetylacetone, 0.5 M, was the most effective reagent, extracting 94% of the total carbon.

Extraction of humic materials from natural waters has

been carried out by methods of adsorption on adsorbents such as activated carbon and alumina and silica gels. Recoveries from these adsorbents are usually low. It has been found that high recoveries of organic compounds from natural waters are possible on macroporous XAD resins (Weber and Wilson, 1975; Ishiwatari et al., 1980; Aiken et al., 1979; Mantoura and Riley, 1975; Maggi et al., 1984). Aiken et al. (1979) studied five XAD resins and determined the capacities and elution efficiencies of the resins. The acrylic ester resins (XAD-7 and XAD-8) had higher adsorption capacities for fulvic acid and were more easily eluted than the styrene divinyl benzene resins (XAD-1, XAD-2 and XAD-4). XAD-2 resin was studied by Mantoura and Riley (1975) to determine the optimum conditions for extraction. Adsorption was favoured at low pH ($\underline{c}$ 2), and desorption was achieved most efficiently with 0.2 M NaOH. Comparatively high recoveries (91%) were also achieved with aqueous methanol-1.0 M ammonia. It was found practical to load the resin to only 20% of its theoretical adsorptive capacity, otherwise significant leakage occurred due to the large volumes of water passing through the columns.

Recently, the efficiency of these XAD resins has been utilised in an extraction procedure developed for the recovery of fulvic acids from soils (Gregor and Powell, 1986a). Fulvic acids are released from the soil by elution with 0.1 M pyrophosphate at pH 2. Following a series of concentration steps, the fulvic acids are separated from the extractant and co-extracted metal ion impurities by adsorption on XAD-7 resin. The organic matter is recovered from the resin at pH 6.5, and converted to its protonated form by cation exchange. High recoveries (> 98%) from the resins are reported, and the resulting acids have low ash contents (typically < 0.6%). Detailed studies of the XAD-7 resin, and other potentially

useful resins, are discussed in Chapter 6.

1.5.2 *Critical Assessment of Extraction Procedures*

Although alkali extraction has been widely used for the recovery of fulvic acids from soils, this method has been criticised. Unfortunately, not only does the alkali release fulvic acids, it also releases humic acids which have to be separated. Further, at high pH phenolic materials are extremely susceptible to atmospheric oxidation (Mihailovic and Cekovic, 1971). Complete removal of oxygen from the soil solution prior to alkali treatment is difficult. Even in the absence of oxygen alteration of organic matter can occur from reactions such as ester hydrolysis. Further, under acidic conditions (used to precipitate humic acids and commonly as a pretreatment to remove polyvalent metal ions) phenols and some hydroxy carboxlyic acids may be altered by a redox reaction with ferric ions. In the presence of iron (III) salts at pH < 6 polyphenols are rapidly and irreversibly oxidised to quinones (Powell and Taylor, 1982), themselves reactive speices.

Tinsley and Salam (1961) have criticised the alkali method, believing that the extracted material would be modified. Autoxidation of the organic matter in contact with the air, and other chemical changes such as condensation of amino acids with carboxyl groups of aromatic aldehydes or quinones may occur. Evidence to support this was reported by Bremner (1950) who observed an increase in alkalinity of the extractant solution. He reported that the more alkaline the solution and the longer the extraction period, the greater was the change. Swift and Posner (1972) demonstrated that some breakdown of humic substances could take place under alkaline conditions. Although treatment with alkali under

N_2 produced some changes (reduction in molecular weight, hydrolytic release of amino acids) the changes were much greater under oxidative conditions. Other changes occurred in the oxidising conditions that did not occur under N_2. Large increases in oxygen content with concomitant increases in C=O and -COOH, and decreases in C-H were observed. Extensive conversions of acid-insoluble to acid-soluble material occurred.

Other workers have reported evidence that suggested extraction with alkali would not modify the organic matter. Forsyth (1947) reported that there was no differences in properties between fulvic acid extracted with NaOH or with water. Schnitzer and Skinner (1968) extracted fulvic acid with 0.5 N NaOH and with 0.1 N HCℓ and found no distinct differences between the extracts in ultimate, functional group and infrared analyses, nor in fractionation by gel chromatography.

During acid treatment to lower the inorganic content, prior to alkali treatment, lower molecular weight fulvic acid fractions may be released and lost from the extract. Similarly, lower molecular weight fractions may be lost from the extract during dialysis procedures. To avoid the loss of such fractions, Holtzclaw et al. (1976) used repeated high speed centrifugation to remove colloidal iron and aluminium, instead of dialysis.

Although the use of organic solvents lead to efficient recoveries of organic matter, Schnitzer and Khan (1972) suggested that organic solvents may lead to extensive alteration of the humic materials by incorporation of carbon and nitrogen from the extractants. It was also a problem to effectively remove all traces of the solvent from the final solution.

Fulvic acids isolated by alkali methods are commonly reported to have ash contents of 2 to 10%, whereas those recovered by XAD resins have lower ash contents, 1 to 2% (Thurman, 1985). When fulvic acids are used in metal complexing studies, it is essential that the acids are low in inorganic components (Fe(III), Aℓ(III) and silicon). High inorganic ash contents result in unstable pH readings during potentiometric titrations, and to errors in total metal concentrations.

1.6 OBJECTIVES OF THIS STUDY

This thesis presents a study of fulvic acid, a specific fraction of soil humus. The ability of this organic matter to bind with protons and metal ions was studied to gain further insight into its composition and structural features.

The complex nature of fulvic acid makes it difficult to study using the standard characterization techniques applied to organic ligands,e.g. nmr, ultraviolet, visible and infrared spectroscopy. Also, the usual techniques applied to the study of proton-ligand and metal-ligand equilibria (e.g. potentiometric pH and ion-selective electrode titrations, ultraviolet or visible spectroscopy) do not allow calculation of protonation and stability constants for such a complex mixture of ligands. Commonly information must be inferred from the study of simpler, but chemically similar, ligands.

Chapter 5 presents a study of the reactions between aluminium(III) and protons with the citrate ion. Aluminium(III) is a dominant metal ion in soils, and the citrate ion, being a polydentate-polycarboxylate ligand, has been considered as a model ligand for fulvic acids. A study of the aluminium(III) citrate equilibria was therefore considered relevant to the theme of this study. Further, it allowed one to gain

expertise in studying metal-ligand equilibria and to become familiar with the development of chemical models and computer programs suitable for the interpretation of the more complex fulvic acid systems. These numerical and computational methods are presented in Chapter 2. A critical assessment of previous studies of the aluminium-citrate equilibria is included in Chapter 5.

For studies on fulvic acids to be meaningful, it is essential that the acids are not modified during their extraction from soils. The traditional methods of extraction, involving both strongly alkaline and strongly acidic solutions are likely to lead to artifacts. Chapter 6 discusses refinements which were made to the previously developed pyrophosphate extraction procedure. Specifically, a further study of resins that could be utilized in the separation of the extractant from the dissolved organic matter was undertaken.

Chemical and physical properties (e.g. elemental analyses; ash contents; molecular weight distributions; nmr, ultraviolet, visible and infrared spectra) of soil fulvic acids, extracted using the refined acid-pyrophosphate method, were studied. Chapter 7 includes the characterization of a variety of fulvic acids extracted from different sources. The effects of alkali treatment on the properties of fulvic acids are also discussed.

The acid-base properties of fulvic acids are discussed in Chapter 8. The titration curves are interpreted in terms of a mixture of monoprotic acids. Numerical analysis of pH titration data generated protonation constants for the various monoprotic acids, and their respective concentrations. The validity of this modelling approach was assessed by the interpretation of data from titrations of mixtures of model ligands.

The ability of the fulvic acids to complex with cations

such as copper(II) and lead(II) was studied (Chapter 9). No attempt was made to determine stability constants for these ligands. However, the complexing ability of fulvic acids from different sources was compared with that of simpler model ligands, e.g. citric, malonic, phthalic, salicylic acids. They were compared on the basis of formation curves, i.e. % free metal vs. pH curves for titration of solutions at fixed metal and ligand concentrations. Techniques including ion selective electrode titrations, fluorescence quenching and polarographic analyses were used. The applicability of these various techniques to indicate the metal complexing ability of fulvic acid was assessed by comparing the formation curves generated by each technique.

Polarographic studies, including ASV, cyclic voltammetry and pulse polarography, yielded information about the effects of adsorption of organic matter on the electrode surface. Information concerning the lability of copper(II)- and lead(II)-fulvic acid complexes was gained from ASV studies. Processes that affect the observed current (e.g. adsorption, lability, mobility) were resolved into two components; those occurring during the plating (reduction) step, and those occurring during the stripping (oxidation) step.

CHAPTER 2

NUMERICAL AND COMPUTATIONAL METHODS

This chapter outlines the necessary equations and computational methods required to determine ligand protonation constants and metal-ligand stability constants from pH titrations on ligand-proton and metal-ligand equilibrium systems.

Part A. NUMERICAL METHODS

Part A of this chapter is concerned with developing equilibrium expressions for the reactions of polyprotic bases with protons and metal ions. It also includes discussions on the correction of computed protonation constants for variations in ionic strength which occur during a titration, and on the determination of protonation constants for mixtures of monoprotic acids. These equations form the basis of the user's subroutines PRELIM and CALC in the general least squares computer program discussed in Part B. Stoichiometric expressions for titration end point determinations are discussed in Section (2.3). A brief outline of the theory of regression analysis, as it was applied to the determination of protonation and stability constants from titration data, is also presented.

2.1 LIGAND PROTONATION REACTIONS

2.1.1 *Polyprotic Acids*

When a weak acid is titrated with a strong base the acid is deprotonated in steps to generate a series of conjugate bases described by the equilibria

$$LH_n \Leftrightarrow LH_{n-1} + H \qquad (2.1)$$

(charges are omitted for clarity). The equilibrium protonation constants, K_n, are defined by the equation

$$K_n = \frac{[LH_n]}{[LH_{n-1}][H]} \qquad (2.2)$$

Alternatively, the cummulative protonation constants, β_i, are defined for the reaction

$$L + i\,H \Leftrightarrow LH_i,\ \beta_i = \frac{[LH_i]}{[L][H]^i} \qquad (2.3)$$

The solution composition can be expressed in terms of stoichiometric (total) concentrations of each species, or in terms of equilibrium concentrations. The total concentration of ligand in solution (T_L) is the sum of the equilibrium concentrations of all ligand-containing species, i.e.

$$T_L = [L] + [HL] + \ldots + [H_nL] \qquad (2.4)$$

Similarly, the total concentration of titratable protons in solution (T_H) is the sum of the equilibrium concentrations

of all proton-containing species, i.e.

$$T_H = [H] + [HL] + \ldots + n[H_nL] - [OH] \qquad (2.5)$$

The 'degree of protonation' of a ligand at any point in a titration, $\bar{n}_H$, is defined as the concentration of protons bound divided by the total ligand concentration, i.e.

$$\bar{n}_H = (T_H - [H] + [OH])/T_L \qquad (2.6)$$

or, by substitution of equation (2.5) into (2.6)

$$\bar{n}_H = ([HL] + 2[H_2L] + \ldots + n[H_nL])/T_L \qquad (2.7)$$

The equilibrium [OH] arises from the hydrolysis of conjugate bases, e.g. $L^{3-} + H_2O \Leftrightarrow HL^{2-} + OH^-$.

T_H and T_L can be determined from acid-base titration data.

If THI and TLI are the initial concentrations of titratable protons and ligand respectively, and Q is the dilution factor at any given point in a titration, then

$$T_H' = THI \times Q$$

$$T_L' = TLI \times Q$$

and

$$T_H = T_H' - ALK$$

Thus, $\bar{n}_H$ can be calculated from experimentally derived quantities, and is referred to as $\bar{n}_H(obs)$, i.e.

$$\bar{n}_H(obs) = (T_H' - [H] - ALK + HYDR)/T_L' \qquad (2.8)$$

where ALK is the concentration of protons neutralized by the added alkali (ALK = [KOH] x titre volume/total volume).

The equations for T_L (2.4) and T_H (2.5) can also be written in terms of the variables [H], [L] and K_n (or β_i). Thus, $\bar{n}_H$ can be expressed as a function of the equilibrium protonation constants and the hydrogen ion concentration. This form of $\bar{n}_H$ is referred to as $\bar{n}_H(calc)$, i.e.

$$\bar{n}_H(calc) = \frac{K_1[H] + 2K_1K_2[H]^2 + \ldots}{K_1[H] + K_1K_2[H]^2 + \ldots} \qquad (2.9)$$

To obtain the K_n values, a non-linear least squares analysis is performed on titration data, minimizing the quantity $\Sigma(\bar{n}_H(obs) - \bar{n}_H(calc))^2$, where the sum is over all data points. This analysis is described in detail in Sections 2.4 and 2.5. The subroutines used in the FORTRAN computer program ORGLS are listed in Appendix A.

As a specific example, consider the case of a triprotic acid (H_3L), e.g. citric acid; the equilibrium protonation reactions are

$$H + L \Leftrightarrow HL \qquad K_1 = [HL]/[H][L]$$
$$H + HL \Leftrightarrow H_2L \qquad K_2 = [H_2L]/[H][HL]$$
$$H + H_2L \Leftrightarrow H_3L \qquad K_3 = [H_3L]/[H][H_2L]$$

The mass balance equations for T_H and T_L are

$$T_H = [H] + [HL] + 2[H_2L] + 3[H_3L] - [OH] \qquad (2.10)$$

and

$$T_L = [L] + [HL] + [H_2L] + [H_3L] \qquad (2.11)$$

or, expressed as a function of the equilibrium protonation constants

$$T_H = [H] + K_1[H][L] + 2K_1K_2[H]^2[L] + 3K_1K_2K_3[H]^3[L] - [OH] \qquad (2.12)$$

and

$$T_L = [L] + K_1[H][L] + K_1K_2[H]^2[L] + K_1K_2K_3[H]^3[L] \qquad (2.13)$$

These two equations, when substituted into equation (2.6), define the $\bar{n}_H(calc)$ function, i.e.

$$\bar{n}_H(calc) = \frac{K_1[H] + 2K_1K_2[H]^2 + 3K_1K_2K_3[H]^3}{1 + K_1[H] + K_1K_2[H]^2 + K_1K_2K_3[H]^3} \qquad (2.14)$$

2.1.2 *Correction for Variation in Ionic Strength*

Thermodynamic protonation constants (K_n^0) are expressed in terms of the activities (a_n) of the reactants and products. The activity of a species is defined as the product of its concentration and activity coefficient (γ_n), i.e. for the equilibrium

$$H + H_{n-1}L \Leftrightarrow H_nL \ ,$$

$$K_n^0 = \frac{a(H_nL)}{a(H)\,a(H_{n-1}L)} = \frac{[H_nL]}{[H][H_{n-1}L]} \cdot \frac{\gamma(H_nL)}{\gamma(H)\,\gamma(H_{n-1}L)} \qquad (2.15)$$

$$= K_n \cdot \frac{\gamma(H_nL)}{\gamma(H)\gamma(H_{n-1}L)} \qquad (2.16)$$

where K_n, a product of concentrations, is the equilibrium protonation constant. Unlike K_n^0, K_n is not a constant except at constant ionic strength. It varies as γ_n varies with ionic strength (Davies equation, $-\log \gamma_n = Az^2(I^{1/2}/(1+I^{1/2})-bI$; Davies, 1962). Thus most equilibrium measurements are made in the presence of a high concentration (0.1 - 1 M) of an inert electrolyte. However, during a titration small changes in ionic strength do occur. The effect of these changes on $\log K_n$ will be greatest at low concentration of inert electrolyte and can be assessed by use of equation (2.16) and the definition of γ_n. At ionic strength I,

$$\log K_n = \log K_n^0 + \phi \qquad (2.17)$$

where ϕ is the activity coefficient term in equation (2.16) expressed in the form of the Davies equation, i.e.

$$\begin{aligned}\phi &= -\log \gamma(H_nL) + \log \gamma(H) + \log \gamma(H_{n-1}L) \\ &= A(I^{1/2}/(1+I^{1/2})) \times (z_{H_nL}^2 - z_H^2 - z_{H_{n-1}}^2) \qquad (2.18)\end{aligned}$$

A similar equation ($\log K_n'$) can be written for the ionic strength I' at any given datum point in the titration. It follows that

$$\log K_n' - \log K_n = \phi' - \phi$$

or,

$$K_n' = K_n \cdot 10^{(\phi'-\phi)} \qquad (2.19)$$

Thus, the protonation constant at nominal ionic strength I, corrected for variation in ionic strength, can be calculated by replacing K_n with K_n', equation (2.19), in the $\bar{n}_H$(calc) equation for the least squares refinement of the $\bar{n}_H$ function.

2.1.3 *Mixtures of Monoprotic Acids*

It is well known that the titration curve of a diprotic acid resembles that of a mixture of two monoprotic acids if the K_n values are sufficiently well separated (Simms, 1926). Perdue et al. (1984) calculated the titration curve for benzenehexacarboxlyic acid and compared it to the calculated titration curve for a mixture of six monoprotic acids that had pK_a' values corresponding to the six pK_a values of the hexaprotic acid. On the basis of the pH titration data alone, it was not possible to distinguish the polyprotic acid from the mixture of monoprotic acids. From this, it follows that the titration curve of a polyprotic acid may be interpreted in terms of a mixture of n-monoprotic acids with "titration" constants,

$$K_n' = [HA]/[H][A] \qquad (2.20)$$

These "titration" constants (K_n') are not numerically equal to the protonation constants of the polyprotic acid (K_n), because the $(n-1)^{th}$ protonation step for the latter may not be complete when the n^{th} one begins. For monoprotic acids, the protonations

$$A^- + H^+ \rightarrow HA$$

$$B^- + H^+ \rightarrow HB \quad \text{etc.}$$

are all independent, whereas for the polyprotic acid the protonation of one conjugate base (say H_2L^-) is dependent on the preceding protonation step, viz.

$$H_2L^- + H^+ \rightarrow H_3L$$

depends on the formation of H_2L^- from

$$HL^{2-} + H^+ \rightarrow H_2L^-$$

This is shown diagramatically in Figure 8.1. However, if the constants are sufficiently different ($K_{n-1} > 100\ K_n$) then K_n' will be approximately equal to the corresponding protonation constant, K_n (equation (2.2)).

To explore the validity of this interpretation of protonation reactions, the "titration" constants of citric acid (assumed to be 3 monoprotic acids) were determined and compared to the protonation constants of the triprotic acid.

To effect this calculation the $\bar{n}_H$(calc) function (equation (2.14)) was replaced with equation (2.27) which was derived as follows:

$$T_H' = [H] + [HA] + [HB] + [HC] - [OH] \qquad (2.21)$$

and

$$T_L' = [HA] + [A] + [HB] + [B] + [HC] + [C] \qquad (2.22)$$

where [HA], [HB] and [HC] correspond to [HL], $[H_2L]$ and $[H_3L]$ of the triprotic acid but represent independent weak acids.

$$\bar{n}_H(\text{calc}) = \frac{[HA] + [HB] + [HC]}{[HA] + [A] + [HB] + [B] + [HC] + [C]} \qquad (2.23)$$

The "titration" constant expressions, equations (2.24), (2.25) and (2.26),

$$H + A \Leftrightarrow HA \qquad K_1' = [HA]/[H][A] \qquad (2.24)$$

$$H + B \Leftrightarrow HB \qquad K_2' = [HB]/[H][B] \qquad (2.25)$$

$$H + C \Leftrightarrow HC \qquad K_3' = [HC]/[H][C] \qquad (2.26)$$

were substituted into equation (2.23) to generate the $\bar{n}_H(\text{calc})$ function in terms of three monoprotic acids, with "titration" constants K_1', K_2' and K_3';

$$\bar{n}_H(\text{calc}) = \frac{K_1'[H][A] + K_2'[H][B] + K_3'[H][C]}{[A](1+K_1'[H])+[B](1+K_2'[H])+[C](1+K_3'[H]} \qquad (2.27)$$

The corresponding total concentrations of HA(=[HA]+[A]), HB(=[HB]+[B]) and HC(=[HC]+[C]) were also generated during the calculation of the "titration" constants.

2.2 METAL-LIGAND EQUILIBRIA

Thermodynamic stability constants for a metal-ligand reaction are defined with respect to the activities of the uncomplexed reactants and of the product species, i.e.

$$pM^{n+} + qH^{+} + rL^{m-} \Leftrightarrow (M_pH_qL_r)^{pn+q-rm} \quad (2.28)$$

$$\beta_{pqr} = \frac{a(M_pH_qL_r)}{a(M)^p . a(H)^q . a(L)^r} \quad (2.29)$$

For experiments made at constant ionic strength, activity coefficients can be assumed to be constant and can thus be incorporated into the stability constant. Alternatively they can be calculated by use of the Davies equation (Davies, 1962)

$$\log \gamma = Az^2(I^{1/2}/(1 + I^{1/2}) - b) \quad (2.30)$$

where A and b are constants, A being characteristic of the solvent; z is the charge on the ionic species; I is the ionic strength

$$I = \Sigma 0.5\ z^2\ [Z] \quad (2.31)$$

If the activity coefficients are assumed to be constant, then the activities of the equilibrium species in equation (2.29) can be replaced by concentrations. These concentration constants are valid only for the conditions at which they are determined.

To determine the stability constants for metal-ligand equilibria (equation (2.29)), the concentrations of species in the equilibrium expression must be determined, either directly or indirectly. Methods including polarography, spectrophotometry, fluorimetry and use of ion selective electrodes have been used to measure the concentrations of free metal or free ligand in such systems. Alternatively,

equilibrium hydrogen ion concentrations can be readily determined by use of a glass electrode.

To study such an equilibrium system, a solution containing metal ions and ligand was titrated with alkali, and the pH monitored as a function of titre. As the titration proceeds the ligand is deprotonated and may complex with the metal. In order to calculate stability constants from potentiometric titration data, a series of mass balance equations must be solved;

$$T_M = [M] + \Sigma p[M_pH_qL_r] \qquad (2.32)$$

$$T_L = [L] + \Sigma r[M_pH_qL_r] \qquad (2.33)$$

and

$$T_H = [H] + \Sigma q[M_pH_qL_r] - [OH] \qquad (2.34)$$

These equations can be rewritten in terms of stability constant expressions, and will yield equations of the form

$$T_H = fn(\beta_{pqr}, K_n, T_L, T_M, [H]) \qquad (2.35)$$

i.e. the unknown stability constants are a function of the stoichiometric concentrations of metal, protons and ligand, the protonation constants of the ligand, and the equilibrium hydrogen ion concentration.

In the mass balance equations for T_L, T_M and T_H, the unknown quantities are [M], [L] and β_{pqr}. An iterative procedure must be applied to solve for [M] and [L] so that they may be substituted into the T_H mass balance equation (2.35). An initial trial value for [L] was estimated from

ligand protonation constants by a rearrangement of equation (2.4)

$$[L] = T_L \; / \; (1.0 + K_1[H] + K_1K_2[H]^2 + \ldots), \qquad (2.36)$$

i.e. assuming no metal complex formation. This trial value of [L] was then used to solve equation (2.32) for [M] using a Newton Raphson iterative method. The value for [M] was then used to solve equation (2.33) for an improved value of [L]. The refined value of [L] was compared with the previous value of [L] (calculated from equation (2.36) for the first cycle, or from equation (2.33) in subsequent cycles); if the two values differed by more than a preselected amount ($\log[L]_{old} - \log[L]_{refined} > 0.001$) the refinement was repeated, i.e. [M] was recalculated from equation (2.32) and substituted into equation (2.33). When the refinement of [L] was adequate, the values of [L] and [M] were used to calculate values for the T_H mass balance equation, i.e. $T_H(calc)$.

An equation for T_H can be set up for each datum point from the experimental quantities pH, T_H' and ALK, i.e. $T_H(obs)$. A non-linear least squares analysis procedure is then used to minimize the function $\Sigma(T_H(obs) - T_H(calc))^2$ over all datum points, and to provide the best values for β_{pqr} (see Sections 2.4 and 2.5). The subroutines used in the FORTRAN computer program ORGLS are listed in Appendix B.

2.3 TITRATION END POINT DETERMINATIONS

Gran Plots

A titration of a weak acid with a strong base usually results in a poorly defined end point, which is not readily estimated by a plot of ΔpH/Δvolume vs. volume. This method relies on accurate data in the region of the end point, a region in which substantial changes in pH occur over a very small titre volume range. The method of end point determination by Gran Plots (Gran, 1952) does not rely on data close to the end point. Titration data at regular intervals before or after the end point are used, especially from the well buffered regions of the titration curve.

For the titration of V mℓ of a weak acid with v mℓ of a strong base (concentration, C),

$$[H] = K_a[HA]/[A] \qquad (2.37)$$

The equilibrium concentrations can be expressed as functions of V,v,C and v_e (the end point titre).

$$10^{-pH} = \frac{K_a(v_e-v)C/(V+v)}{vC(V+v)} \qquad (2.38)$$

or

$$v.10^{-pH} = K_a(v_e-v) \qquad (2.39)$$

Therefore, a plot of $v.10^{-pH}$ vs. v will tend to zero as v tends to v_e, i.e. as the alkali titre tends to the end point titre.

After the end point ($v_e > v$),

$$[OH] = \frac{K_w}{[H]} = \frac{K_w}{10^{-pH}} = \frac{(v-v_e)C}{(V+v)} \qquad (2.40)$$

Similarly, a plot of $K_w/10^{-pH}$ vs. v will tend to zero as v tends to v_e.

2.4 REGRESSION ANALYSIS

Regression analysis is concerned with the problem of estimating the mean value of one variable (dependent variable) on the basis of other variables (independent variables). When only one independent variable is involved, the analysis is termed linear regression. Multiple regression involves more than one independent variable (Harnett, 1982).

2.4.1 *Linear Least Squares Regression*

To specify the line y = bx + a, which defines the linear relationship between the dependent variable (y) and the independent variable (x), the slope (b) and intercept (a) need to be defined. The mean value of y can be related to the actual value of y (y_i) for a given value of x (x_i) by e_i, the random error

$$e_i = y_i - \bar{y} \qquad (2.41)$$

The procedure for finding the best estimates of a and b is called 'the method of least squares'. The procedure involves finding the values of a and b so that the resulting values of y are as close as possible to the actual values of y_i, i.e. minimize equation (2.41). It is usual to determine the values of a and b which minimize the sum of the squared errors, i.e. minimize

$$\sum_{i=1}^{n} e_i^2 = \sum_{i=1}^{n} (y_i - \bar{y})^2 \qquad (2.42)$$

This is equivalent to minimizing

$$\sum_{i=1}^{n} [y_i - bx_i - a]^2 \qquad (2.43)$$

In order to minimize this expression, it is necessary to take the partial derivative with respect to the variables concerned (a and b), to set each of these partial derivatives equal to zero, then solve the equations simultaneously. This yields a line with slope

$$b = \frac{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2} \qquad (2.44)$$

and intercept

$$a = \bar{y} - b\bar{x} \qquad (2.45)$$

In minimizing equation (2.42), the method automatically sets $\sum e_i = 0$, i.e. the positive residuals (underestimates) always exactly cancel the negative residuals (overestimates).

2.4.2 *Nonlinear Least Squares Regression*

As was the case for linear regression, a relationship between independent variables (x_i) and dependent variables (y_i) is required. Consider the relationship $y = a + bx + cx^2$. The equation to be minimized would be

$$\sum_{i=1}^{n} (y_i - a - bx_i - cx_i^2)^2 \qquad (2.46)$$

The partial derivatives with respect to a, b anc c are set equal to zero and solved simultaneously using matrix algebra to yield values for a, b and c.

Part B. COMPUTER PROGRAMS

Part B of this chapter presents a variety of computer programs used in this study of metal-ligand and proton-ligand interactions. It includes an outline of the procedure for the least squares program adopted for the analysis of acid-base titration data, and discusses the computer programs used to generate distribution diagrams for polyprotic ligands and their metal complexes.

2.5 GENERAL LEAST SQUARES PROGRAM

The General Least Squares FORTRAN program used in this work (adapted from program ORGLS of W.R. Busing & H.A. Levy, Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1962) adjusts parameters of a defined function (Y) to obtain the best least squares fit to a set of observations. The function used is defined by a set of parameters in subroutine CALC, Y(calc), and is also defined in a second subroutine (PRELIM) by a set of independent variables (experimental observations), Y(obs). The observations and trial parameters are read prior to entering subroutines PRELIM and CALC so that they may be passed in to the subroutines for data manipulation and function evaluation.

Subroutine PRELIM is entered once at the beginning of the program. This subroutine is used to read in various

constants, define auxillary functions, manipulate input data (e.g. pH calibration corrections), and to calculate the value of the observed function, Y(obs), (e.g. $\bar{n}_H$(obs), T_H(obs)) for each datum point.

In any one cycle of refinement, the subroutine CALC is entered once for each observation to obtain a value of Y(calc), generated from the current parameters.

Y(obs) and Y(calc) are stored in arrays and passed back to the main program where a least squares analysis is performed. The program cycles NC times, incrementing the current parameters each cycle, recalculating Y(calc), and re-evaluating the least squares fit. The refinement terminates when either NC cycles are complete, or there is no further improvement in the fit, as determined by a convergence test. After the refinement of parameters is complete, subroutine CALC is entered for a final time to calculate Y(calc). A list comparing the observed and calculated function, and an agreement factor, is printed before each cycle and again after the final refinement. After each cycle the current parameters and their new improved values are listed.

Examples of the user's subroutines PRELIM and CALC are listed in Appendices A and B. Appendix A contains the subroutines for determination of protonation constants for a triprotic acid. Appendix B contains the subroutines for determination of stability constants for a metal-ligand system.

2.6 DISTRIBUTION DIAGRAMS

The composition of ligand-proton solutions and metal-ligand solutions can be calculated as a function of pH provided protonation constants and metal-ligand stability constants are known.

2.6.1 *Ligand Composition*

The composition of a ligand solution can be calculated at any pH by solving equation (2.3) for the individual LH_i. [H] can be calculated from pH and [L] can be calculated from equation (2.36). A computer program was written to calculate the fraction of T_L that LH_i represents, as a function of pH (see Appendix C).

2.6.2 *Metal-Ligand Composition*

Appendix D is a FORTRAN computer program written to calculate the composition of a copper-ligand solution. Similar programs have been written for iron and aluminium complexes. The program cycles through, once for each pH increment. Within each cycle, a series of loops are performed to estimate [M], allowing for all possible complexes of k competing ligands. Initially, [L] for each ligand is estimated from equation (2.36), and used to approximate [M]. Then, this value of [M] is used to redefine the values of [L] by a Newton Raphson iterative method. From the refined values of [L], [M] is recalculated, and is substituted into stability constant expressions to solve for $[M_pH_qL_r]$.

CHAPTER 3

EXPERIMENTAL

3.1 VOLUMETRIC EQUIPMENT

All pipettes used in quantitative work were calibrated by dispensing and weighing aliquots of water. The weight (w) of water dispensed was converted to a volume (v) by use of the equation $v = w/\delta$, where δ is the density of water at ambient temperature as given in the CRC Handbook of Physics and Chemistry (1981).

The volumetric flasks used in this work were B-grade, and were assumed to have errors in total volume as given by Vogel (1961).

3.2 PREPARATION OF SOLUTIONS

3.2.1 *NBS Buffers*

The buffer solutions used to define the pH response of the glass/calomel electrode combination were : 0.05 M tetroxalate, 0.05 M potassium hydrogen phthalate, 0.025 M disodium hydrogen phosphate/0.025 M potassium dihydrogen phosphate, and 0.01 M sodium tetraborate. These buffers are assigned pH values of 1.679, 4.008, 6.865 and 9.183 respectively, at 25° C (Bates, 1973).

Each buffer was prepared by dissolving the calculated weight of pure, dry solid in 2 ℓ of doubly distilled water.

3.2.2 *Standard Acid and Alkali Solutions*

(a) Standard HCℓ

A stock solution of HCℓ (c 1 M) was prepared by dilution of concentrated Analar HCℓ with Milli-Q deionised water. The solution was standardized by titration against known weights of Na_2CO_3 (dried to 350°C). The concentration of the stock HCℓ was determined as 1.089 ± 0.004 M.

(b) Standard HNO_3

A stock solution of HNO_3 (c 1 M) was prepared by dilution of concentrated Analar HNO_3 with Milli-Q deionised water. The solution was standardized, by titration against known weights of anhydrous Na_2CO_3, as 0.937 ± 0.006 M.

(c) Standard KOH

Two stock solutions of KOH (c 1 M) were prepared during the course of this work. The solutions readily take up CO_2 from the atmosphere, forming insoluble carbonates which alter the hydroxide concentration.

The solutions were prepared by carefully eliminating possible sources of carbonate contamination. Firstly, an excess (c 30%) of Analar KOH pellets was washed rapidly with freshly boiled Milli-Q deionized water that had been nitrogen saturated. This removed the carbonate surface from the pellets. The pellets were then dissolved in carbon dioxide free Milli-Q deionized water.

Solutions were standardized by replicate titration against known weights of potassium hydrogen phthalate. Stock solutions of 0.961 ± 0.003 M and 0.970 ± 0.006 M were prepared.

Dilutions of standard acid and alkali solutions were prepared in Milli-Q deionized water. For alkali dilutions, the water was freshly boiled and nitrogen saturated.

3.2.3 *Electrolyte Solutions*

1 M solutions of KNO_3 and KCℓ were prepared from calculated weights of Analar salts dissolved in Milli-Q deionized water.

3.2.4 *Ligand Solutions*

(a) Citric Acid

Standard citric acid solutions (c 0.05 M) were prepared by dissolving known weights of $H_3L.H_2O$ in Mill-Q deionized water. The concentrations were confirmed by titration with standard KOH. Dilutions of the standard solutions were made for potentiometric titrations (Chapter 5); solutions of 2.53×10^{-3}M and 2.02×10^{-3}M were prepared in 0.1 M KCℓ.

For ^{13}C nmr studies, a 2 M solution of citric acid and a 2 M solution of trisodium citrate were prepared by dissolving a calculated weight of the substance in doubly distilled water. Solutions, 1 M in ligand, were prepared by dilution and adjusted to pH 1.0, 3.5, 4.7, 8.0, 11, 14, 15 and 15.3, by the addition of HCℓ or KOH. At high pH (14-15), a series of 1 M ligand solutions was also prepared at constant ionic strength (I = 11 M) by addition of LiCℓ.

(b) Tricarballylic Acid

Solutions of similar compositions to those described for citrate were prepared for ^{13}C nmr studies of the

tricarballyate ion. 1 M solutions were prepared and adjusted to pH 2.0, 3.4, 5.2, 7.0, 9.5, 11.2, 13.8 and 15.3. Solutions were also prepared at constant ionic strength (I = 11 M) in LiCℓ at pH 14.0, 14.4, 14.6 and 14.7.

(c) Fulvic Acid

Fulvic acid is a hygroscopic material. Drying a sample to constant weight, and maintaining that constant weight during weighing is difficult without appropriate precautions. Following the method of Campbell (1968), fulvic acid samples were dried in capsules made from aluminium foil. The fulvic acid samples, sealed in aluminium capsules, were dried at 78°C (over refluxing ethanol), under vacuum for 2 h.

Fulvic acid solutions (c 16 mg/250 mℓ) were prepared with and without added electrolyte. The solutions used for potentiometric titrations were in 0.1 M KCℓ, whereas solutions for conductivity titrations contained no added electrolyte.

Ligand solutions for ion selective electrode potentiometry and polarographic studies were c 12 mg/50 mℓ.

Fulvic acid solutions, 1.0 mg/mℓ, in 0.01 M borax buffer, were used for Sephadex G50 chromatography.

(d) Miscellaneous Ligand Solutions

Standard phthalate, malonate and salicylate solutions 5.0×10^{-3}M, 8.9×10^{-4}M and 7.3×10^{-5}M respectively, were prepared for ion selective electrode and fluorescence quenching studies (Chapter 9). The solutions were standardized by titration against standard KOH.

A mixed ligand solution, 2.0×10^{-4} M in phthalic acid and 3.56×10^{-4}M in malonic acid, was prepared in 0.1 M KCℓ for potentiometric titration (Chapter 8).

3.2.5 *Metal Solutions*

(a) Aluminium(III)

A previously standardized solution of $AlC\ell_3$ (Kennedy, 1984) was used throughout this study for quantitative work. The solution was 0.1007 M in 0.01 M HCℓ.

For aluminium(III) - citric acid ^{13}C nmr studies, a 1.00 M Al^{3+} solution in 0.1 M HCℓ was used.

(b) Copper(II)

$Cu(NO_3)_2.3H_2O$ (recrystallized from HNO_3) was dissolved in 1.88×10^{-3} M HNO_3. This standard Cu^{2+} solution (0.050 M) was further diluted to generate 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M solutions. The dilute solutions were prepared in 0.1 M KNO_3 as background electrolyte, and HNO_3 was added to give a final pH of 4.1 (to prevent formation of copper hydroxy species).

3.2.6 *Metal-Ligand Solutions*

(a) Aluminium(III) - Citrate Solutions

For potentiometric studies on the aluminium(III) - citrate equilibrium system, three solutions were used:

(i) (Al^{3+} : citrate) 1 : 6.34
citric acid, 2.55×10^{-3} M
Al^{3+}, 4.028×10^{-4} M
excess H^+, 8.75×10^{-3} M
in 0.0827 M KCℓ.

(ii) (Al^{3+} : citrate) 1 : 6.34
citric acid, 1.02×10^{-3} M
Al^{3+}, 1.611×10^{-4} M
excess H^{+}, 3.50×10^{-3} M
in 0.093 M KCℓ.

(iii) (Al^{3+} : citrate) 1 : 5.45
citric acid, 2.55×10^{-3} M
Al^{3+}, 4.683×10^{-4} M
excess H^{+}, 4.65×10^{-5} M
in 0.094 M KCℓ.

^{13}C nmr spectra were recorded for solutions of Al^{3+} (0.25 M) and citrate (1 M) at pH 2.0, 4.0, 4.6, 5.1, 6.0 and 8.1. These solutions were prepared from a 1.0 M Al^{3+} solution and a 2 M citric acid or 2 M trisodium citrate solution by addition of KOH.

(b) Copper(II) - Ligand Solutions

For ion selective electrode potentiometry and fluorescence quenching studies (Chapter 9), acidic solutions of Cu^{2+} (4.0×10^{-4} M) and ligand (1.8×10^{-4} M COOH) were prepared for titration with standard KOH. The ligands used were citric, aspartic, malonic, salicylic or fulvic acids, prepared in 0.1 M KNO_3. Further fulvic acid solutions at 1 : 20 (Cu^{2+} : COOH) ratio were also prepared, where the Cu^{2+} was 9.0×10^{-6} M.

3.3 SOILS AND FULVIC ACID SAMPLES

3.3.1 *Soils*

Three soil types were used as sources for fulvic acid. Two of the soils lay adjacent to each other in a Maimai gley Podzol, West Coast, South Island, New Zealand. These were a B_h subhorizon and a B_s subhorizon. The third soil was the International Humic Substances Society (IHSS) bog peat.

3.3.2 *Fulvic Acid Samples*

Four fulvic acid samples were extracted from these soils using the pyrophosphate/XAD-7 method (Chapter 6), two coming from B_h subhorizons. The samples will be designated FA1, FA2, FA3 and FA4, corresponding to B_h, B_h, B_s and IHSS sources, respectively.

Three other fulvic acid samples were used in this study. They were :

(i) An alkali extracted fulvic acid supplied by Dr M. Schnitzer (sample designated FAS),

(ii) an acid extracted fulvic acid supplied by Dr H.A. Anderson (FAA), and

(iii) the IHSS reference fulvic acid extracted from a peat (FAI).

The elemental composition of these samples is presented in Table 3.1.

Table 3.1 Fulvic Acid Elemental Composition (%)

	FAS[d]	FAA[d]	FAI
C	43.8	47.6	50.9
H	3.6	4.0	3.26
N	0.6	0.6	2.32
ash[a]	1.2	4.1	2.3
P[b]	0.17	0.59	0.0
Al	0.02	0.25	0.02
Ca	0.02	0.09	0.31
Fe	0.081	0.73	0.03
Si	0.65	0.14	0.17
Na	0.25	0.90	0.44
K[c]	-	0.04	0.07

a : by ignition at 450°C, 2 h

b : by inductively coupled plasma spectroscopy for P, Al, Ca, Fe, Si, Na

c : by flame atomic absorption spectroscopy

d : ref. Cressey et al., 1983

3.4 RESINS

3.4.1 *XAD Resins*

An Amberlite styrene divinylbenzene resin XAD-4 and the acrylic ester resins XAD-7 and XAD-8 (Aldrich Chemical Company), were initially prepared by washing the resin beads with dilute ammonia solution (2 h) and distilled water, followed by hot soxhlet extractions with acetone then

methanol (each 2 h). The resins were stored in distilled water. A sample of XAD-7 resin was further treated by hot soxhlet extraction with dichloromethane (4 h) and methanol (4 h). XAD resins could be regenerated after use by the same procedure.

3.4.2 *Other Resins*

The anion exchange gel, diethylaminoethyl (DEAE) Sephadex was studied for its use in the extraction of soil fulvic acids. It was packed into a glass column (0.6 cm x 6 cm) using ultrasonic vibration and treated with 0.01 M HCℓ prior to use.

Polyethylene Glycol Dimethacrlyate (PGM2000) gel was studied for its use in the extraction of soil fulvic acid. It was packed into a glass column (1 cm x 12 cm) and pretreated with 0.01 M HCℓ.

Sephadex G50 gel chromatography was used to examine molecular weight distributions of fulvic acids. The fulvic acid was dissolved in 0.01 M borax and eluted through a column of the gel (1 xm x 34 cm) which had been pretreated with 0.01 M borax solution to minimize change in composition at the solvent front.

3.5 ANALYTICAL PROCEDURES

3.5.1 *Microanalysis*

Elemental C, H and N analyses of fulvic acid samples were performed at Otago University. Oxygen contents were estimated by difference.

3.5.2 *Inductively Coupled Plasma Spectroscopy (ICP)*

The inorganic ash components of fulvic acid samples were determined by ICP on a Labtest model V25 instrument, by R.W. Finlayson. Known weights of fulvic acid (typically 25 mg) were dissolved in 20 mℓ of distilled water.

3.5.3 *Atomic Absorption Spectroscopy (AAS)*

Flame and carbon furnace AAS were performed on fulvic acid samples. Flame AAS (Varian AA 1475) was used to determine Na and K contents, using standard operating parameters. Carbon furnace AAS (GBC model 902 instrument with GF1000 furnace programmer) was used to determine Fe and Al contents. Injections of 5 μℓ were used. Operating parameters for Fe were : dry - 120^{o} C, 10 s; ash - 700^{o} C, 10 s; atomize - 2400^{o} C, 2 s. Operating parameters for Al analysis were : dry - 100^{o} C, 10 s; ash - 500^{o} C, 10 s; atomize - 2700^{o} C, 2 s.

3.5.4 *Nuclear Magnetic Resonance (NMR)*

^{13}C nmr spectra were recorded for aqueous solutions of citrate and aluminium - citrate at 32^{o} C on a Varian CFT-20 nmr spectrometer (100 MHz). Typically, 3000-10000 transients were collected with a 1 s data aquisition time and flip angle of 3^{o}. Chemical shifts were referenced to an external dioxan standard at 67.4 ppm.

Temperature dependent (3 - 63^{o} C) ^{13}C nmr spectra of aluminium -citrate solutions were recorded on a Varian XL300 spectrometer (300 MHz), measuring 100 transients with 0.9 s aquisition time.

^{13}C and ^{1}H nmr spectra of fulvic acid solutions were recorded on a Varian CFT-20 nmr spectrometer. Fulvic acid

(80 mg) was dissolved in 0.4 mℓ of D_2O. Typically 1000 transients were collected for ^1H nmr spectra, and > 10^6 transients for ^{13}C.

3.5.5 *Ultraviolet and Visible Absorption Spectroscopy*

Ultraviolet and visible absorption spectra were recorded on a Varian DMS100 spectrophotometer. Ultraviolet or visible absorption spectroscopy was used in conjunction with chromatographic work to quantify phthalic acid (274-278 nm), catechol (275 nm), epicatechin (275 nm), an epicatechin polymer (274 nm), fulvic acid (465 nm or 300 nm), and phosphate as the phosphomolybdate complex (800 nm).

3.5.6 *Infrared Spectroscopy*

1 mg samples of fulvic acid and 500 mg of KBr were pressed into discs and scanned from 4000 to 200 cm^{-1} on a Pye Unicam SP3-300 Infrared spectrophotometer.

3.5.7 *Gℓc Analysis*

Gℓc traces of fulvic acid alkaline hydrolysis products extracted into acetone were recorded using a Shimadzu GC 9A instrument with SPB-5 glass capillary column (30 m x 0.75 mm ID). The temperature program used was : ramp 100-250° C at 10° min^{-1}), then hold 250° C.

3.5.8 *Fluorimetry*

An EEL fluorimeter, with an OX1 primary filter isolating 360 nm excitation radiation, and a 622 secondary filter isolating 460 nm emission radiation, was used to study fluorescence quenching of fulvic acid solutions in the presence of copper(II) (Chapter 9).

3.6 POLAROGRAPHIC TECHNIQUES

3.6.1 *Instrumental Parameters*

A Princeton Applied Research Model PAR174 polarograph, with a model 303 dropping mercury electrode and a Houston Omnigraphic 2000 X-Y recorder, was used for all polarographic measurements.

The polarograph was used in several modes with both dropping mercury electrode (DME) and hanging mercury electrode (HME). In the DME mode, metal solutions were scanned using sampled dc, normal pulse and differential pulse polarographic techniques. Unless otherwise stated, the drop time was 0.5 s, scanning from +0.2 V at 10 mV/s.

In the HME mode, cyclic voltammetry and anodic stripping voltammetry (sampled dc and differential pulse) were used. Cyclic voltammograms were recorded with a scan rate of 100 mV/s, and an initial potential of -0.4 V. Deposition for ASV experiments was carried out on a 'medium' size mercury drop, usually at -0.9 V, for 60-240 s. The voltage was scanned at 10 mV/s during the stripping process.

3.6.2 *Experimental Procedures*

All polarographic experiments were carried out in a Class 100 clean room. Solutions were prepared in either 0.01 M acetate buffer (pH 4.8) or in 0.02 M $HCl O_4$ (pH 1.7). Socorex micropipettes were used to dispense volumes less than 1 mℓ.

Solutions were flushed with oxygen-free nitrogen for 12 minutes prior to measurements.

Copper(II) solutions were prepared by dilution of a 2.5×10^{-5} M solution. Lead(II) solutions were prepared by

dilution of a 4.0×10^{-5} M stock solution. A combined Cu/Pb/Cd solution, with each metal 1.2×10^{-5} M, was also used. For ASV experiments, typical metal concentrations were 1.25×10^{-7} M, and typical ligand concentrations were 9×10^{-6} M COOH (diluted from $\underline{c}$ 12 mg/50 mℓ solutions).

CHAPTER 4

POTENTIOMETRIC TITRATIONS

This chapter has been divided into two parts. Part A discusses the equipment and experimental procedures used for titrations involving either a glass electrode, a copper(II) ion selective electrode, or a conductivity probe. In part B, the theory of calibration of a glass electrode/calomel electrode pair as a hydrogen ion concentration probe is briefly discussed, and the experimental procedures presented. The Nernstian response of an ion selective electrode is discussed and the experimental procedures for its calibration are described.

PART A. EXPERIMENTAL

4.1 GENERAL TITRATION ASSEMBLY

4.1.1 *Cell Design*

Two titration cells, with maximum capacities of 120 mℓ and 50 mℓ, and minimum operating capacities of 100 mℓ and 40 mℓ respectively, were used. The minimum capacities were limited by the volume of solution required to fully immerse the electrodes. These cells were double-walled to allow external circulation of thermostated water at 25.0 $\pm$ 0.1^{o}C (Figure 4.1). Airtight lids were fitted to the titration cells by means of silicon greased ground glass flanges on both the lid and the base. The lids

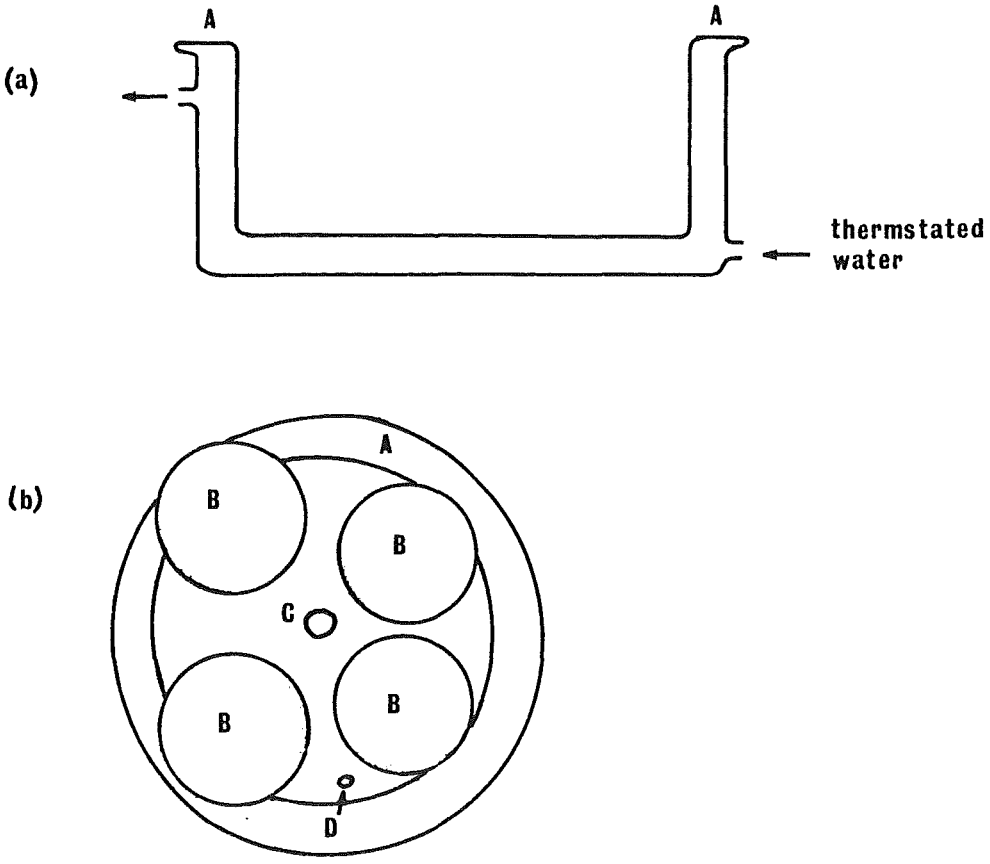


Figure 4.1 Thermostated titration cell, (a) cross section through cell, (b) plan view of lid. A, ground glass flange; B, quick-fit ports for electrodes; C, quick-fit port for nitrogen bubbler; D, port for alkali syringe tip.

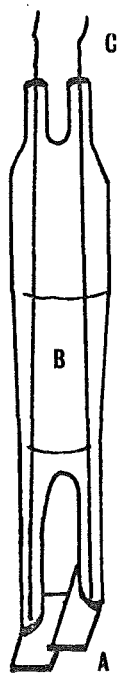


Figure 4.2 Conductivity probe.
A, parallel platinum plates;
B, quick-fit joint;
C, electrode terminals.

had several ground glass ports to allow airtight inclusion of electrodes, a nitrogen bubbler and a syringe tip. Correspondingly, all electrodes, the nitrogen bubbler and the syringe tip were either fitted with ground glass collars or sealed into rubber stoppers.

4.1.2 *Oxygen Removal*

Rigorous exclusion of oxygen from titration solutions was achieved by purging them with oxygen-free nitrogen. Nitrogen was released from the titration cell via a water trap, to completely seal the cell.

The oxygen-free nitrogen was further purified prior to entry into the titration cell by passing it through an acidic vanadium(II) solution to remove dissolved oxygen, then through an alkali solution to remove dissolved carbon dioxide. The vanadium(II) oxygen scrubber consisted of an acidified solution of V(II) over a Zn/Hg amalgam (Taylor, 1980). The Zn/Hg amalgam was prepared by stirring Zn granules in a 2% solution of $HgC\ell_2$ in 1 M $HC\ell$, thus coating the Zn with Hg. The excess $HgC\ell_2$ was removed by repeated washing of the amalgam with distilled water. The vanadium solution was prepared from a solution of 0.45 g $VOSO_4.2H_2O$ in 50 mℓ of 2 M H_2SO_4. The V(IV) was reduced by the Zn/Hg amalgam and the colour changed from blue, through green to violet as the oxidation state of the vanadium changed from +(IV) to +(III) to +(II).

4.1.3 *Burettes*

To accurately dispense standard alkali and acid titrants, Gilmont digital micrometer syringes were used.

The syringes were fitted with 2.5 mℓ glass burette tips. The syringes are graduated in 0.001 mℓ increments with vernier to 0.0001 mℓ. Where possible, the total titrant volume dispensed was approximately 2 mℓ to minimize titre errors. The syringes, one each for the acid and alkali solutions, were calibrated by dispensing and weighing aliquots of water. The weight was converted to volume by use of the known density of water (CRC Handbook of Physics and Chemistry, 1981).

4.2 ELECTRODES AND POTENTIOMETERS

4.2.1 *Glass Electrodes*

For accurate pH measurements (± 0.003 pH) a Beckman E-2 glass electrode (type 39004) and a Radiometer calomel electrode (type K401) were coupled with a Radiometer PHM64 Research pH meter. Occassionally it was necessary to use a Beckman Research pH meter.

For less accurate pH measurements, a Beckman general purpose glass electrode (type 41263) was used.

The Beckman E-2 glass electrode and calomel electrode combination were calibrated against solutions of known hydrogen ion concentration in 0.10 M KCℓ. This calibration procedure is discussed in Section (4.4). The hydrogen ion calibration procedure was performed only once for a given pair of electrodes, and was referenced to NBS buffers. For daily use, the electrodes need only be standardized relative to the NBS buffers.

4.2.2 *Conductivity Probe*

Conductivity titrations were performed with a specially designed conductivity probe. The probe consisted

of two parallel platinum plates, each supported and protected by glass rods (Figure 4.2). The electrode was fitted with a ground glass collar so that it could be inserted into the thermostated titration cells used for pH titrations.

4.2.3 *Ion Selective Electrodes*

A Radiometer Ruzicka Selectrode (model F3012) ion selective electrode was made copper(II) sensitive by applying a layer of electroactive copper Selectrode powder (S42015) to the conducting surface. The electrode was initially conditioned in 0.1 M EDTA overnight, then stored either in distilled water, if in daily use, or in 0.1 M EDTA for long term storage.

The copper(II) ion selective electrode is sensitive to halide ions. Therefore, halide solutions could not be used as supporting electrolytes. Chloride contamination from the reference calomel electrode was minimized by separating the reference electrode from the titration solution with a solution of non-interfering KNO_3 (0.1 M). The KNO_3 was contained within a glass jacket fitted around the calomel electrode. The tip of the jacket was fitted with a vycor frit.

The copper(II) ion selective electrode was standardized for daily use against standard copper(II) solutions. This procedure is discussed in more detail in Section (4.5).

4.3 TITRATION PROCEDURES

Many of the procedures used in the potentiometric analyses were common to pH, ion selective electrode and conductivity titrations, these routine procedures will be

discussed in Section (4.3.1). Procedures specific to ligand titrations or metal-ligand titrations will be treated separately in Sections (4.3.2) and (4.3.3) respectively. Details of solution compositions are given in Chapter 3.

4.3.1 *General Procedures*

Titrations were always performed in the thermostated cells described in Section (4.1.1) at 25°C. The majority of the titrations were performed in the larger (120 mℓ) cell. The smaller cell was used for some fulvic acid titrations where ligand had to be used sparingly.

The first step in any titration was to standardize the electrodes. The pH electrode pair was standardized against tetroxalate, phthalate, phosphate and borax (NBS) buffers, at 25°C. The ion selective electrode pair was standardized against two standard copper solutions (usually 10^{-2} and 10^{-4} M), in the presence of acidified ligand.

The solution to be titrated was pipetted into the titration cell. The electrodes, syringe tip and nitrogen bubbler were inserted, and the cell sealed to the atmosphere. At least 30 minutes was allowed for the temperature to reach equilibrium and for the solution to become nitrogen saturated. The titrant was then dispensed into the solution, in aliquots such that 60 - 100 datum points were recorded evenly throughout a pH titration, or 20 - 40 datum points for the ion selective electrode studies. Sufficient time was allowed for equilibrium to be established after each addition of titrant. By the time the potentially oxygen sensitive region for a fulvic acid titration was reached (pH > 6.5), the solution had been purging with nitrogen for at least 60 minutes, ensuring complete removal of oxygen.

At the completion of the titration, the electrodes were again standardized to allow correction for drift in electrode response with time.

4.3.2 *Ligand Titrations*

Ligand solutions were titrated for three reasons:

(i) To accurately determine the concentration of the solution.

(ii) To determine conductivity titration end points.

(iii) To collect data for analysis in terms of protonation reactions.

Standardization of ligand solutions required titration with a standard KOH solution to determine the end point titre. Ligands standardized by titration included citric, malonic, phthalic, salicylic and aspartic acids. The concentrations of fulvic acid solutions were also determined by titration, and were expressed as equivalent weights, i.e. (moles of titratable acid groups)/weight of fulvic acid.

Conductivity titration curves reflect the changes in concentration of species with differing charges and specific conductivities. Consequently, these titrations were performed in the absence of added electrolyte. Conductivity titrations and pH titrations were carried out simultaneously. To avoid chloride contamination from the pH reference electrode, the electrode was jacketed with the ligand solution being titrated. Chloride ions diffusing out of the reference electrode were essentially retained in the jacket solution.

To determine the protonation constants for fulvic acid (or citric acid) from pH titration data it was necessary to

collect data over a wide pH range. Titration with standard KOH solution generated data up to, and beyond, the carboxyl end point for fulvic acid (pH 7.9), although data above pH 7.0 were rarely used in calculations because of the larger error associated with data near the end point. To generate data below the initial pH, identical solutions to those titrated with alkali were titrated with standard HCℓ to protonate the more strongly acidic carboxyl groups. Data below pH 2.7 were rarely used in calculations because of the large uncertainty in $(T_H - H)$ at low pH (see Section 2.1.1, equation 2.8).

Solutions of citric acid, a mixture of malonic and phthalic acids, and fulvic acid were titrated to generate data for numerical analysis. The results of the citric acid titrations are reported in Chapter 5. Results for fulvic acid and the malonic acid/phthalic acid mixture are discussed in Chapter 8.

4.3.3 *Metal - Ligand Titrations*

(a) Aluminium(III) - Citric Acid

The equilibrium reactions between aluminium(III) and citrate ions were determined by pH potentiometry. Titrations of two types were used:

(i) Titration with standard KOH into solutions at fixed metal and ligand concentrations.

(ii) Titration of ligand solution with aluminium(III) ions at fixed pH.

The titration of a citrate solution with aluminium(III) ions was performed at pH 8.7. Following each addition of Al(III),

the pH dropped. The released protons were then titrated with standard KOH to re-establish the pH to 8.7. From this titre, the proton stoichiometry of the aluminium(III) - citrate complex was determined. Results from the aluminium(III) - citrate titrations are reported and discussed in Chapter 5.

(b) Copper(II) - Ligand

To study the interaction of Cu(II) ions with ligands such as citric, malonic and fulvic acids, copper(II) ion selective electrode potentiometry was used. Solutions, 1.8×10^{-4} M in ligand carboxyl groups and either 4.0×10^{-5} M or 9.0×10^{-6} M in Cu(II), were titrated with standard KOH from pH 4.0 to pH $\underline{c}$ 7.3, then back-titrated with standard HCℓ to pH 2.8. The emf was monitored as a function of pH. From the emf of the Cu electrode the % free metal was calculated. The supporting electrolyte was 0.1 M KNO_3; KCℓ could not be used because the ion selective electrode is sensitive to halide ions. The ligands studied by ion selective electrode potentiometry were citric, aspartic, malonic, phthalic and salicylic acids. These ligands were selected as model ligands to compare results with those for copper(II) - fulvic acid complexing. The results of these studies are reported in Chapter 9.

PART B. ELECTRODE CALIBRATION

4.4 Calibration of a Glass Electrode as a Hydrogen Ion Concentration Probe

The equilibrium concentration quotient for a ligand protonation reaction, $H_{n-1}L + H \Leftrightarrow H_nL$, is defined by (see Section 2.1.1),

$$K_n = \frac{[H_nL]}{[H_{n-1}L][H]}$$

Similarly, the equilibrium concentration quotient for the reaction, $pM + qH + rL \Leftrightarrow M_pH_qL_r$, is (see Section 2.2),

$$\beta_n = \frac{[M_pH_qL_r]}{[M]^p[H]^q[L]^r}$$

Such equilibria are commonly followed by potentiometric titration, using a glass electrode/calomel electrode combination.

To determine the values of K_n or β_n, the equilibrium hydrogen ion concentration, [H], is required for use in solving the mass balance equations. However, the pH electrode pair measures the activity of hydrogen ions, not the concentration. Also, the emf for the electrode pair will include an undefined liquid junction potential term.

4.4.1 *Hydrogen Ion Activity*

By definition, $pH = -\log a_H$, where a_H is the activity of the hydrogen ion. The activity of an ion is related to its concentration by,

$$a_x = [X]\gamma_x \qquad (4.1)$$

where γ_x is the activity coefficient of the ion X. Thus, to determine the hydrogen ion concentration from pH, the activity coefficient of the hydrogen ion is required. Although single ion activity coefficients cannot be measured, they can be calculated by equations such as the Debye-Hückel equation or

the Davies equation (equation 2.30). However, these equations are only valid for dilute solutions of a single electrolyte solution.

4.4.2 *Liquid Junction Potentials*

For a standard buffer, the emf of the glass electrode/ calomel electrode pair is given by,

$$E(s) = E' + E_{as} + E(s)_{LJ} + pH(s)(2.303RT/F)$$

where E' is related to the difference in emf of the internal and external reference electrodes, E_{as} is the asymmetry potential of the glass membrane, and E_{LJ} is the liquid junction potential for the calomel electrode.

Similarly, for a solution of unknown acidity,

$$E(x) = E' + E_{as} + E(x)_{LJ} + pH(x)(2.303RT/F)$$

Thus, the pH of the unknown solution can be related to the pH of the standard solution and the measured emf by,

$$pH(x) = pH(s) + \frac{(E(s) - E(x)) + (E(x)_{LJ} - E(s)_{LJ})}{2.303RT/F}$$

However, only if the liquid junction potentials of the unknown and standard solutionsare the same will the measured pH of the unknown solution correspond with the pH scale on which the standard is defined. This will seldom be the case.

A liquid junction potential arises across a junction between two electrolyte solutions of different composition, e.g. a liquid junction potential arises between the standard or

unknown solution and the filling solution of the reference electrode. Although the electrolyte of the reference electrode filling solution will remain constant, the composition and ionic strength of the unknown solution will probably not match those of the standard buffer solutions.

Thus, in general, calibration against NBS buffers will involve an error in pH because $E(x)_{LJ} \neq E(s)_{LJ}$. Further, a direct measure of $[H^+]$ is not afforded.

4.4.3 *Determination of p[H] at Constant Ionic Strength*

The two problems discussed in Sections (4.4.1) and (4.4.2) can be countered by calibrating the electrode assembly against standard solutions of known hydrogen ion concentration, [H], at the same ionic strength and composition as the unknown solution (Bates, 1973). At constant ionic strength, the activity coefficient of the hydrogen ion will be nearly the same in both the standard and unknown solutions, and thus the residual liquid junction potential will be small.

Standards in the pH range 2 to 4 and 10 to 12 can be generated from solutions of strong acids (e.g. $HC\ell$, $HC\ell O_4$) and strong bases (e.g. KOH). In the intermediate range (pH 3 to 11), buffer systems have been used. These may be used to generate a solution of fixed [H], or to generate a family of solutions of known [H] by titration against standard alkali or acid.

Very few buffer systems for electrode calibration have been reported; accurate concentration quotients at selected ionic strengths are required. Hedwig and Powell (1971) used ethylenediamine - ethylenediammonium buffers for the pH range 4 to 10. The acetic acid - acetate buffer is

often used in the pH range 3.8 to 5.0. Kennedy et al. (1983) reported the use of the o-phthalic acid - phthalate system for standard buffers in the pH range 3.0 to 5.4.

p[H] calibrations are standardized against the NBS buffers (see Section 4.4.4). On a day-to-day basis only the NBS buffer response need be checked.

4.4.4 *NBS Buffers*

The pH of a solution (x) is defined relative to a standard solution (s). The standard is usually one of the standard NBS (National Bureau of Standards) buffers. These standard reference buffers have pH values assigned from measurements on cells without liquid junction. A series of buffers are defined as primary pH standards. These buffers, of specific concentration, are stable in solution and have a pH value between 3 and 11. The seven primary reference pH standards presently recognized by IUPAC are:

(i) saturated (at 25^{o}C) potassium hydrogen tartrate.

(ii) 0.1 M potassium dihydrogen citrate.

(iii) 0.05 M potassium hydrogen phthalate.

(iv) 0.025 M disodium hydrogen phosphate/0.025 M potassium dihydrogen phosphate.

(v) 0.03043 M disodium hydrogen phosphate/ 0.008695 M potassium dihydrogen phosphate.

(vi) 0.01 M sodium tetraborate.

(vii) 0.025 M sodium hydrogen carbonate/0.025 M sodium carbonate.

pH values for the primary standards, at a variety of temperatures, are well documented (Covington et al., 1983; Bates, 1973).

These seven primary NBS buffers show internal consistency between pH 3.5 and 11, i.e. the measured pH of a solution will be the same regardless of which of the seven buffers is used as the standard. Outside this pH range the measured pH will deviate from the defined scale due to changes in liquid junction potentials (Bates, 1973). Powell and Taylor (1983) have measured the pH of buffer solutions in cells with and without liquid junctions. The liquid junction term was approximately constant between pH 4 and 9.2, but lead to deviations in measured pH outside this range. For example, for 0.05 M tetroxalate buffer, pH = 1.639 in a cell with liquid junction (and pH = 1.687 in a cell without liquid junction); potassium hydrogen phthalate pH = 4.005 (4.005); carbonate/bicarbonate pH = 9.989 (10.019). Thus in cells with liquid junctions it is not possible to determine pH values below 4 or pH above 9.2 by extrapolation beyond the range of the seven primary NBS buffers. Allowance must be made for the pH-dependence of the liquid junction at low or high pH.

4.4.5 *Calibration Procedures*

In this work a Beckman E-2 glass electrode (type 39004)/Radiometer calomel electrode (type K401) combination was calibrated to measure hydrogen ion concentration in the pH range 1.7 to 5.3. Solutions of known hydrogen ion concentration were generated by titration of (i) standardized HCℓ solutions with KOH, and (ii) a phthalic acid solution with KOH, at constant ionic strength (I = 0.1 M KCℓ).

Two HCℓ solutions, 0.0216 M and 0.0108 M, were titrated with standardized KOH (0.961 M) to generate data in the pH range 1.7 to 2.5. A solution of 0.005 M phthalic

acid was also titrated with 0.961 M KOH, generating data in the pH range 2.5 to 5.3.

Calculation of p[H] for the phthalic acid titration required an iterative procedure. Concentration quotients at I ≈ 0.10 M (Q_1, Q_2) were calculated from the published dissociation constants (I = 0.0) using an extended Debye-Hückel expression for single ion activity coefficients (Hamer et al., 1945). The value of [H] was determined for each datum point, from mass balance equations and initial approximations of [H](= 10^{-pH_m}), I, Q_1 and Q_2, by the Newton-Raphson method. The calculated value of [H] was used to estimate solution composition and improved values of I, Q_1 and Q_2. These improved values of I, Q_1 and Q_2 were then used to estimate [H]. The iterative process terminated when the change in I was less than 0.005 mol kg^{-1} (Kennedy et al., 1983).

The measured pH (pH_m) was related to p[H] by the relationship pH_m = 1.013 p[H] + 0.04137. This relationship was derived from a linear least squares analysis of titration data. The calibration line generated for HCℓ titration data was found to be the same as that for the phthalic acid titration data within experimental error.

4.5 CALIBRATION OF A COPPER(II) ION SELECTIVE ELECTRODE

4.5.1 *Nernstian Response*

An important property of any ion selective electrode should be Nernstian response over the widest possible concentration range, i.e.

$$E = E^{o} + \frac{2.303RT}{nF} \log a_i$$

where E is the potential of the ion selective electrode with respect to the reference electrode, E^{o} is the standard potential of the assembly, 2.303RT/nF is the Nernst factor, and a_i is the activity of the ion in solution.

It should be noted that E depends on the activity of the ion, not its concentration. The activity of an ion can be related to its concentration by equation (4.1). However, the single ion activity coefficient (γ_i) has to be calculated from a empirical equation such as the Davies equation (equation 2.30). This calculation is dependent on the ionic strength of the solution, as is the liquid junction potential of the reference electrode. These two ionic strength dependent variables can be kept constant by preparing reference (or standard) solutions at constant ionic strength, commonly 0.1 M. At constant ionic strength, pM is directly proportional to -log [M], and at 25^{o} C a plot of -log[M] vs. E will, (with ideal Nernstian behaviour) have slope 2.303RT/nF (= 59.16/n mV). For the copper(II) ion selective electrode, where n=2, the Nernst factor (or slope) should be 29.58 mV.

4.5.2 *Calibration Procedures*

The copper(II) Selectrode ion selective electrode used in this work was initially calibrated against standard Cu^{2+} solutions, in the range 10^{-2} to 10^{-6} M. The electrode response was further calibrated at lower copper concentrations against copper-citrate buffer solutions.

The standard Cu^{2+} solutions were prepared by dilution of a stock (0.05 M) Cu^{2+} solution. The solutions were prepared in 0.1 M KNO_3 as the supporting electrolyte. HNO_3 was also added to the solutions such that the final

concentration was 7.5×10^{-5} M HNO_3. The acidic conditions (pH 4.1) ensured that copper did not form copper hydroxy species.

The electrode response (E) was found to be linear over the range pM 2 to 6, using standard Cu^{2+} solutions. Although the slope of the calibration line remained constant on a day-to-day basis, the intercept varied slightly, but was typically of the form E(mV) = 30.5 pM - 325.

To extend the calibration to larger values of pM, copper(II) solutions of known $Cu(aq)^{2+}$ concentration were generated by titration of an acidic copper-citrate solution with standard KOH. The copper-citrate system is sufficiently well defined to allow calculation of copper concentrations from its known stability constants for any solution stoichiometry and any pH. Data below pH 4 were corrected for liquid junction effects by standardizing the pH electrode pair against tetroxalate and phthalate buffers. For pH > 4, the electrodes were standardized against phthalate and phosphate buffers. Titration of a solution, 2.56×10^{-3} M citrate and 2.0×10^{-3} M Cu^{2+} in 0.1 M KNO_3, generated copper(II) solutions in the range pM 2.7 to 7.5. Stable emf readings ($\pm$ 0.1 mV) were obtained within 2 minutes. A Nernstian response was established between pM 2 and 7.5.

The calibration line defined by the copper-citrate buffer solution did not coincide with that defined by the standard copper(II) solutions. However, if the standard copper (II) solutions were prepared in the presence of citrate at the same concentration of ligand as in the buffer, and at a pH sufficiently low to prevent complex formation (pH 2.5), then the two calibration lines coincided. It was inferred that citrate had an effect on the electrode

response, possibly through adsorption on the electrode surface. A similar effect was observed when fulvic acid was the ligand.

All subsequent calibrations of the ion selective electrode (for each ligand) were performed with acidic copper(II) solutions in the presence of ligand. On a daily basis, the electrode was standardized against two standard solutions (e.g. pM 2 and 4) in the presence of ligand at pH $\underline{c}$ 2.5.

CHAPTER 5

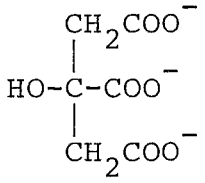
REACTIONS OF CITRATE WITH ALUMINIUM(III) AND PROTONS IN AQUEOUS SOLUTION

5.1 INTRODUCTION

As indicated in Chapter 1, study of the aluminium(III) - citrate system is of relevance to this study. The citrate ion is a polycarboxylate, polydentate ligand. It has been postulated as a suitable model for the soil organic fraction fulvic acid. Aluminium(III) is an important soil cation; as a class A metal ion it has a potential to bind with polycarboxylate ligands such as fulvic acid. Further, the results in the literature on the equilibrium model for the aluminium(III) - citrate system are at variance.

The citrate anion (1) has four potential donor sites; two terminal carboxyl groups, one central carboxyl group and an alkoxy group.

(1)



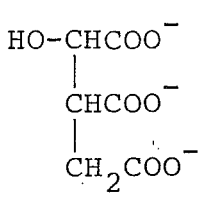
The alkoxy group may co-ordinate in the protonated (—OH) or deprotonated ($—O^-$, alkoxo) form. Because of the geometry of the citrate ion, the maximum number of donors to any one metal centre is three. The possibility then arises that the excess donor can act as a bridging

group, leading to the formation of polynuclear complexes. Carboxylate, alkoxy and alkoxo groups have known tendencies to bridge metal centres. Polynuclear citrate complexes have been established in the solid state for several metal centres: Fe(II) (Strouse et al., 1977), Mn(II) (Carrell & Clusker, 1973) and Mg(II) (Johnson, 1965), and postulated for Cd(II) (Grenthe et al., 1984), Cu(II) (Still & Wikberg, 1980a) and Ni(II) (Still & Wikberg, 1980b).

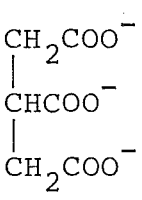
Although most would agree that the citrate ion is tridentate to any one metal centre, there is debate as to which three of the four possible donor groups are involved in coordination. However, coordination of the central carboxyl group, a terminal carboxyl group and the alkoxy group would give the optimium combination of ring sizes. The crystal structure of the Fe(II) - citrate complex $[Fe(H_2O)_6][FeL(H_2O)]_2.2H_2O$ (Strouse et al., 1977) indicates this mode of coordination.

Jackson (1982) compared the stabilities of citrate (1), isocitrate (2) and tricarballylate (3) complexes of Cu(II), Ni(II) and Al(III) and deduced that the two terminal carboxyl groups and the alkoxy group were the donors for Al-citrate.

(2)



(3)



In contrast, Motekaitis and Martell (1984) suggested the involvement of the alkoxo ($—O^-$) group as a donor, along with the central carboxyl group and one of the terminal

carboxyl groups. In the complex AlL, this would require that the non-coordinated carboxyl group is protonated.

5.2 CRITICAL ASSESSMENT OF EQUILIBRIUM MODELS

Several studies of the aluminium(III) - citrate system have been made (Ohman & Sjoberg, 1983; Motekaitis & Martell, 1984; Jackson, 1982; Rajan et al., 1981). However, there remains disagreement between the equilibrium models proposed. Ohman and Sjoberg postulated a model including the mononuclear species $Al(HL)^{+}$, AlL and AlL_2^{3-}, and a trinuclear complex $Al_3(OH)_4L_3^{4-}$. Motekaitis and Martell postulated $Al(HL)^{+}$, AlL and $AlLH_{-1}^{-}$ as the only species present. In addition to the three species proposed by Motekaitis and Martell, Jackson included $AlL_2(OH)^{4-}$.

None of these studies has specifically studied the end point stoichiometry, which would provide information on the upper pH end member species of an equilibrium model. Neither has use been made of any technique, other than potentiometric titration, to establish the presence of specific entities in an equilibrium model.

Ohman and Sjoberg (1983) studied the aluminium - citrate equilibrium at Al^{3+}: citrate ratios of 1:1 to 1:32. However, data from titrations with a large excess of ligand ($\underline{c}$ 10) are not reliable for calculation of stability constants because of the dominant contribution to the total acid mass balance equation from excess ligand. This was reflected in the function Z_c (defined as the average number of hydroxyl ions reacted per citrate ion), plotted as a function of $p[H^{+}]$. Z_c reached limiting values dependent upon the solution stoichiometry; values were close to 3 for citrate: Al^{3+} ratios > 4, and reached a

maximum of 4.35 for equimolar solutions. The limiting value of 4.35 was interpreted as consistent with an end member complex for the 1:1 solution with stoichiometry $Al_3L_3H_{-4}^{4-}$. However, experimental data were restricted, with few data collected above pH 6. It is possible that further proton loss could occur at higher pH for the 1:1 system, and in systems with excess ligand. The same end member species was inferred for solutions with excess ligand; this was based on observed minima in the error square sums obtained for models with different end member species, assuming fixed constants for the other species.

One striking feature of this model is the behaviour of the trimeric species. $Al_3L_3(OH)_4^{4-}$, as a function of pH. It is observed to increase in concentration, then decrease, then again increase with increasing pH. This behaviour is not unknown, but is infrequently reported. Nagypal and Beck (1980) discussed this same behaviour for metal-ligand-proton systems. In these three component systems the complexes formed may either (i) contain titratable protons or be proton deficient, e.g. ML(OH), or (ii) contain no titratable protons, e.g. ML_2. If both types of complex are present over the same pH range, the effect of change in pH may be a concentration minimum for ML(OH) in the pH-dependent distribution curves. Formation of both complexes must involve a common precursor (e.g. ML). A minimum in ML(OH) will occur if the formation of ML_2 removes more of the common percursor (ML) than does the ML(OH) equilibrium over a limited pH range.

Motekaitis and Martell (1984) investigated the equilibrium system at 1:1, 2:1 and 3:1 ratios of citrate: Al^{3+}. The end point titre for a 1:1 solution indicated

neutralization of one proton in addition to those titrated on the tricarboxylic acid, i.e. one additional proton per aluminium. The 2:1 and 3:1 potentiometric curves also indicated the titration of one mole of protons per mole of aluminium excess to the titration of ligand. Results from the 2:1 titration did not allow discrimination between the formation of 1:1 or 1:2 (Al:citrate) complexes, viz. $AlLH_{-1}$ or AlL_2H_{-1}. This arises because at the end point pH of the 2:1 titration (pH 7.5), the value of $\bar{n}_H=0$. Therefore all ligand protons would be titrated at the end point, regardless of whether or not all of the ligand was complexed (as in 2:1 complexes) or only half of the ligand was complexed (as in 1:1 complexes).

At low ratios of ligand:metal severe pH drifting has been encountered in different ligand - Al^{3+} systems, and has lead to poor reproducibility and non-stoichiometric end points (Kennedy and Powell, 1985). Many of the aluminium - citrate studies have been carried out at low ligand:metal ratios. Jackson (1982) used ratios of 1:1 as well as 3:1 and 5:1. Motekaitis and Martell (1984) reported that equilibration times of up to 4 h were required for stable pH readings in 1:1 and 3:1 solutions, whereas times of 6 h were required by Ohman and Sjoberg (1983) at "low" ligand: metal ratios. Kennedy and Powell (1985) found it necessary to work at ligand:metal ratios > 4 to ensure reproducible end point stoichiometries for aluminium - polyphenol interactions.

In this work, a potentiometric and ^{13}C nmr study of the aluminium - citrate equilibria was made. Ligand:metal ratios > 4 were used to avoid pH drifting, the end point stoichiometry was assessed, and ^{13}C nmr studies provided

supporting evidence for the proposed model.

5.3 RESULTS

5.3.1 *Citrate Protonation Constants*

The protonation constants for the citrate ion (L^{3-}) were determined from $p[H^+]$ - volume of titre data for titration of standardized KOH into citric acid solutions, at constant ionic strength and temperature (refer to Chapters 3 and 4 for experimental details). Duplicate titrations were performed at two citrate concentrations, with over 40 datum points collected for each. Data were analysed by a non-linear least squares procedure (see Section 2.4.2), minimizing the function $\Sigma(\bar{n}_H(obs)-\bar{n}_H(calc))^2$ (see Section 2.1.1) over all datum points. Values of log K_n (n = 1-3) are shown in Table 5.1 and the pH dependent distribution curve shown in Figure 5.1. Also shown in this table are results for inclusion of ion pair formation (K^+L^{3-}) in the numerical analysis.

The effect of small changes in ionic strength on log K_n that occur during these titrations ($\underline{c}$ 0.093-0.106 M) was assessed by use of equation (2.19), and is included in Table 5.1.

5.3.2 *Aluminium-Citrate Titrations*

(a) End Point Stoichiometry

The end point stoichiometry of the aluminium-citrate equilibrium system was assessed by two approaches.

Firstly, a solution, 2.5×10^{-3} M in citrate and 2.014×10^{-4} M in Al^{3+} (T_L/T_M 12.7), was titrated with standard KOH. The titration end point (pH 8.3) indicated

Table 5.1 Equilibrium Constants for Protonation of the Citrate (3-) Ion, $25^{o}C$, I = 0.1 M KCℓ.

Reaction	Constant	a	b	c
$L^{3-} + H^{+} \Leftrightarrow LH^{2-}$	log K_1	5.70 ± .02	5.90 ± .02	5.92 ± .01
$LH^{2-} + H^{+} \Leftrightarrow LH_2^{-}$	log K_2	4.35 ± .01	4.35 ± .01	4.35 ± .01
$LH_2^{-} + H^{+} \Leftrightarrow LH_3$	log K_3	2.91 ± .02	2.91 ± .02	2.91 ± .03

mean ± standard deviation for 4 titrations.

a assuming fixed ionic strength (I = 0.1 M KCℓ)

b $K^{+}cit^{3-}$ ion pairing considered

c including correction for variable ionic strength; ion pairing included.

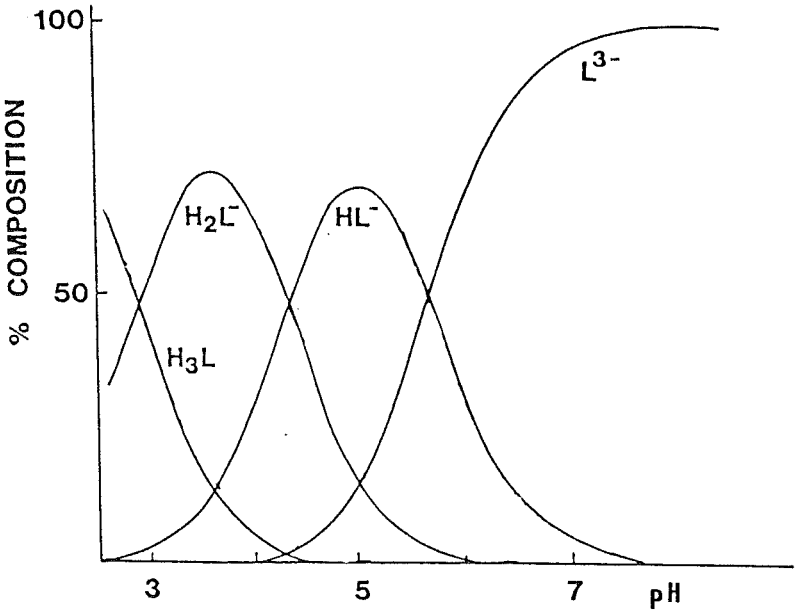


Figure 5.1 Computed solution composition for citrate -H^+ species as a function of p[H^+]; [H_3L] = 2.52 x 10^{-3} M.

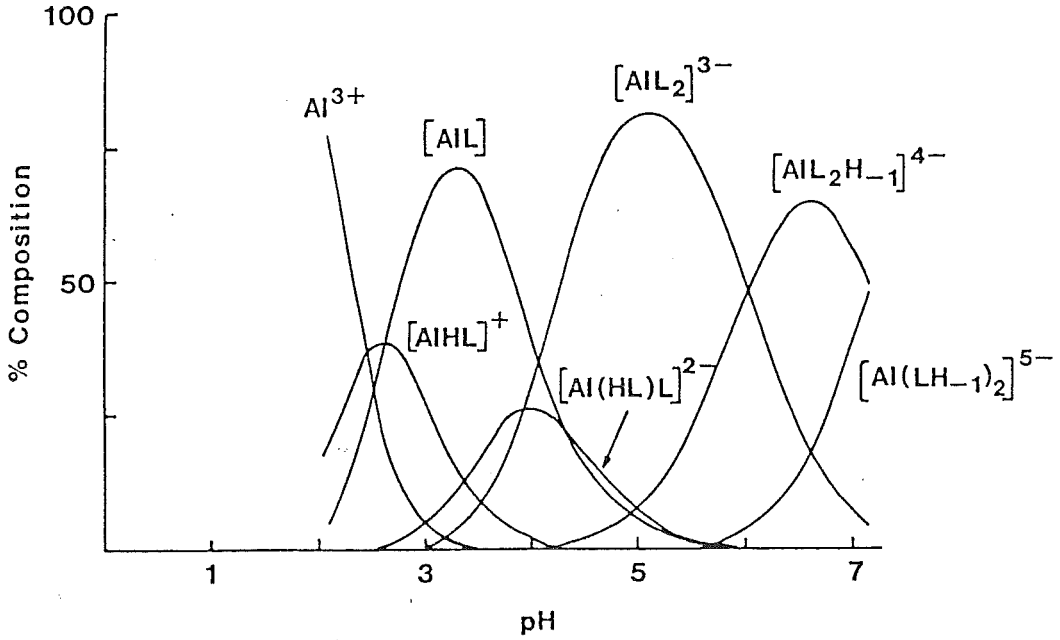


Figure 5.2 Computed solution composition for citrate -Al(III) species as a function of pH; [H_3L] = 2.55 x 10^{-3} M T_L/T_M = 6.3.

release of 3 moles of protons per mole of ligand plus 2.0 $\pm$ 0.2 moles of protons per mole of aluminium.

Secondly, the end point stoichiometry was assessed by titration of the protons released when an Al^{3+} solution (0.10 M) was incrementally added to a citrate solution (2.56×10^{-3} M, pH 8.7, $\bar{n}_H$=0). The mean of 3 experiments, each consisting of $\underline{c}$ 12 datum points, was 2.2 $\pm$ 0.2 moles of protons released per mole of Al^{3+}. Titrations were terminated at $\underline{c}$ T_L/T_M = 15 due to excessive pH drift at low ratios.

The observed end point stoichiometry corresponds to either

$$Al^{3+} + 2L^{3-} \Leftrightarrow Al(LH_{-1})_2^{5-} + 2H^+ \qquad (5.1)$$

or

$$Al^{3+} + 2L^{3-} \Leftrightarrow Al(OH)_2L_2^{5-} + 2H^+ \qquad (5.2)$$

or

$$Al^{3+} + L^{3-} \Leftrightarrow Al(OH)(LH_{-1})^{2-} + 2H^+ \qquad (5.3)$$

One of these end member species must be included in the equilibrium model, along with a sensible route for its formation.

(b) Aluminium(III) - Citrate Stability Constants

Titrations of aluminium - citrate solutions T_L/T_M = 6.34 and T_L/T_M = 5.30, with KOH gave stable pH readings up to pH $\underline{c}$ 7.0 (refer to Chapters 3 and 4 for experimental details). At higher pH, and T_L/T_M < 5, excessive pH drifting occurred.

100 - 150 datum points were collected for each titration, and the data analysed by non-linear least squares regression (see Section 2.4.2) in terms of the following reactions,

$$Al^{3+} + H^{+} + L^{3-} \Leftrightarrow AlHL^{+} \quad (5.4)$$

$$Al^{3+} + L^{3-} \Leftrightarrow AlL \quad (5.5)$$

$$AlL + L^{3-} \Leftrightarrow AlL_2^{3-} \quad (5.6)$$

$$AlL_2^{3-} + H_2O \Leftrightarrow AlL_2H_{-1}^{4-} + H_3O^{+} \quad (5.7)$$

$$AlL_2H_{-1}^{4-} + H_2O \Leftrightarrow Al(LH_{-1})_2^{5-} + H_3O^{+} \quad (5.8)$$

$$Al^{3+} + L^{3-} + LH^{2-} \Leftrightarrow Al(HL)L^{2-} \quad (5.9)$$

The hydrolysis products $Al(OH)^{2+}$, $Al_3(OH)_4^{5+}$ and $Al_{13}(OH)_{32}^{7+}$ were included (Ohman & Forsling, 1981), but made a minor contribution to solution stoichiometry ($\leq$ 0.03%). The computed stability constants are given in Table 5.2 (with and without $K^{+}cit^{3-}$ ion pairing included), and the computed distribution curves are shown in Figure 5.2. Also included in Table 5.2 are the log K values calculated for a single pooled data set (some 570 datum points).

The effect of errors in the concentrations of KOH, aluminium solutions and citrate solutions was assessed. A 0.4% error in KOH concentration resulted in the following uncertainties in log K: $\pm$ 0.07 ($AlHL^{+}$), $\pm$0.09 (AlL), $\pm$ 0.18 (AlL_2^{3-}), $\pm$ 0.22 ($AlL_2H_{-1}^{4-}$), $\pm$ 0.16 ($Al(LH_{-1})^{5-}$). The uncertainty resulting from errors in solution stoichiometry was generally larger than the difference between constants derived from different data sets.

Table 5.2 Equilibrium Constants (log K) for Formation of Aluminium(III) - Citrate Complexes at 25°C, I 0.1M KCℓ.[a]

Reaction	Without K^+cit^{3-} ion pairing	With K^+cit^{3-} ion pairing
$Al^{3+} + H^+ + L^{3-} \Leftrightarrow AlHL^+$	10.81 ± 0.02	11.02 ± 0.02
		11.01 ± 0.01
$Al^{3+} + L^{3-} \Leftrightarrow AlL$	8.10 ± 0.23	8.35 ± 0.23
		8.35 ± 0.01
$AlL + L^{3-} \Leftrightarrow AlL_2^{3-}$	4.80 ± 0.07	5.05 ± 0.12
		5.01 ± 0.01
$AlL_2^{3-} + H_2O \Leftrightarrow AlL_2H_{-1}^{4-} + H_3O^+$	-6.10 ± 0.14	-6.07 ± 0.24
		-6.07 ± 0.01
$AlL_2H_{-1}^{4-} + H_2O \Leftrightarrow Al(LH_{-1})_2^{5-} + H_3O^+$	-7.17 ± 0.02	-7.09 ± 0.08
		-7.18 ± 0.02
$Al^{3+} + L^{3-} + LH^{2-} \Leftrightarrow Al(HL)L^{2-}$	11.14 ± 0.17	11.46 ± 0.09
		11.42 ± 0.02

[a]Mean ± S.D. for 5 titrations. Values in italics refer to calculation based on single pooled data (570) set; R = .35%.

5.3.3 *^{13}C NMR Results*

The pH dependence of ^{13}C chemical shifts for citric acid, tricarballylic acid and aluminium - citrate solutions was determined. The purpose of this study was to establish the pH range in which deprotonation of the citrate alkoxy group occurred when coordinated to Al^{3+}.

(a) Citric Acid Solutions

Citric acid resonances were assigned according to Strouse (1977): A1, -$\underline{C}H_2$-; B1, $\underline{C}$-OH; C1, -$\underline{C}$OOH (terminal); D1, -$\underline{C}$OOH (central) (Figure 5.3). Figure 5.4 shows the chemical shifts of each carbon in citric acid as a function of pH. All resonances shifted downfield between pH 2 and 7, but remained constant from pH 7 to 14. Similar results were obtained for tricarballylic acid.

The effect of high pH (pH 14 - 14.7) on chemical shifts was studied by addition of KOH to citric and tricarballylic acid solutions (Figure 5.5). LiCℓ was added to maintain constant ionic strength . There was no indication of alkoxy deprotonation for citric acid below pH 14.7, i.e. log K > $\underline{c}$ 15.7.

(b) Aluminium(III) - Citrate Solutions

The 100 MHz ^{13}C nmr spectrum of an aluminium - citrate solution is shown in Figure 5.6. Spectra were recorded between pH 2 and 8. The presence of eight resonance lines at pH 2 indicated that complexing commenced below pH 2 and that ligand exchange was slow on the nmr time scale. Four resonances were assigned to free ligand, and the remaining four (A2-D2) assigned to complexed ligand. Figure 5.7 is

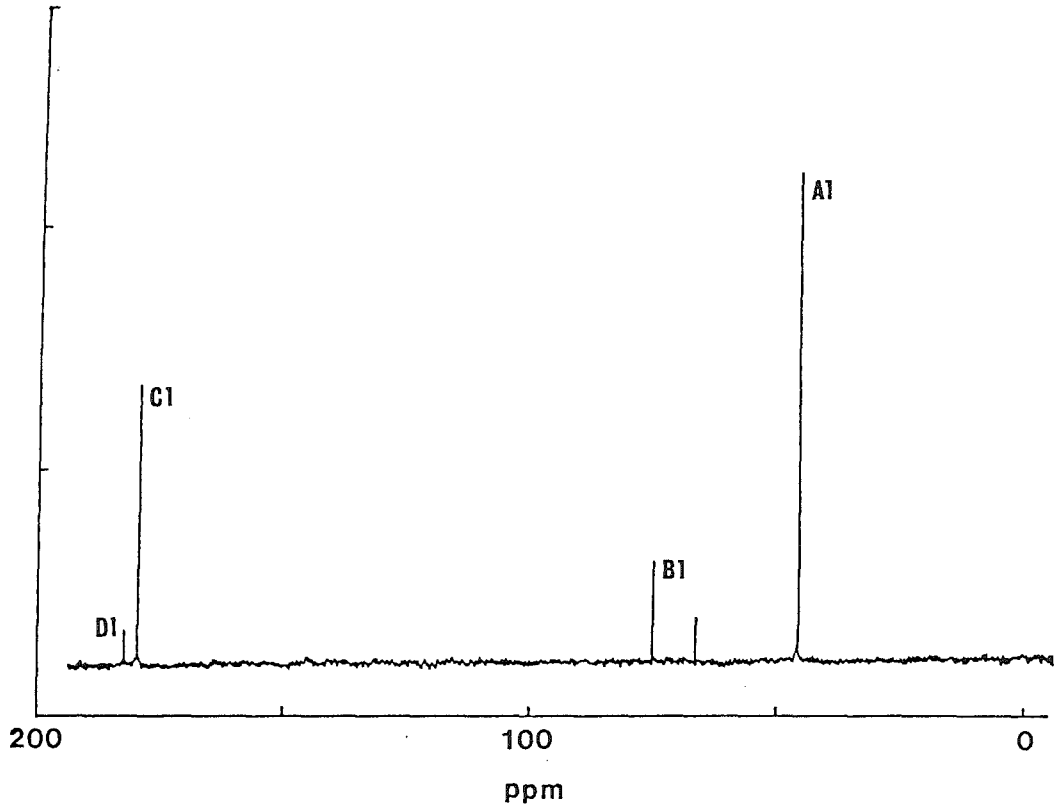


Figure 5.3 (above) ^{13}C nmr spectrum for citric acid pH 6.3, 1.0 M. A1, CH_2; B1, COH; C1, COOH terminal; D1, COOH central.

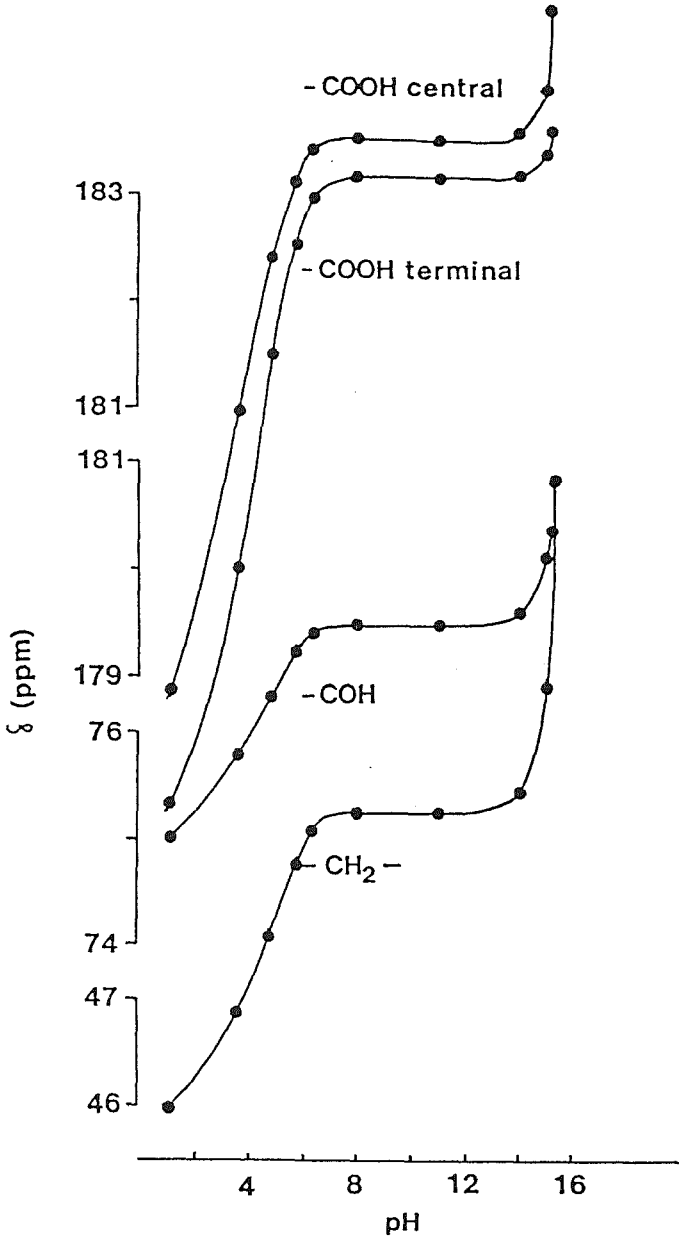


Figure 5.4 (right) ^{13}C nmr chemical shifts for citric acid (1.0 M) as a function of pH (variable ionic strength).

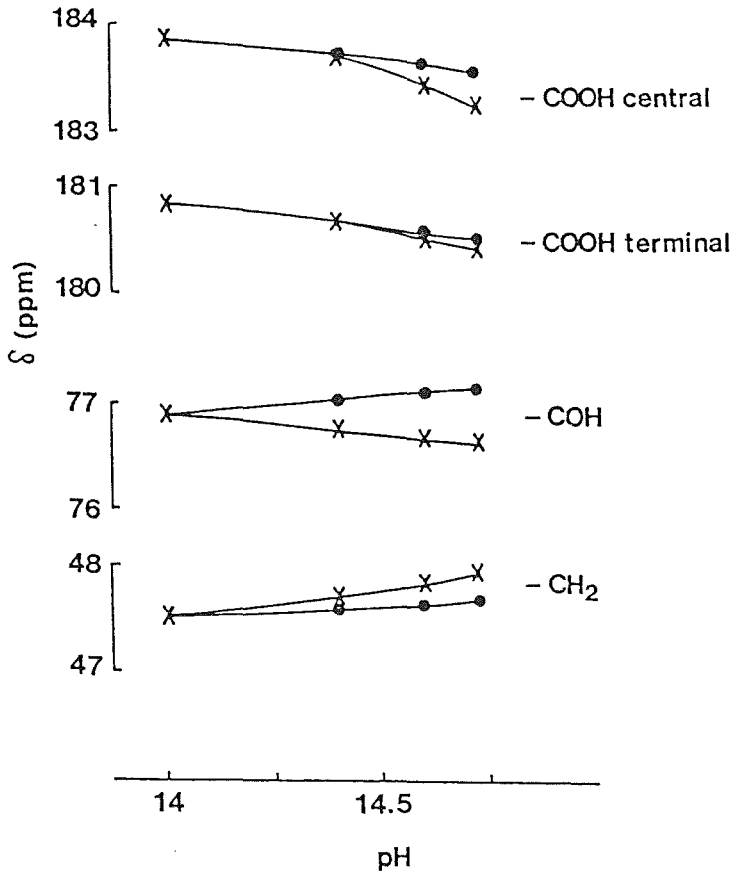


Figure 5.5 ^{13}C nmr chemical shifts at constant ionic strength (11 M LiCℓ), pH 14.0-14.7. x, citric acid; ● propane-1,2,3-tricarboxylic acid.

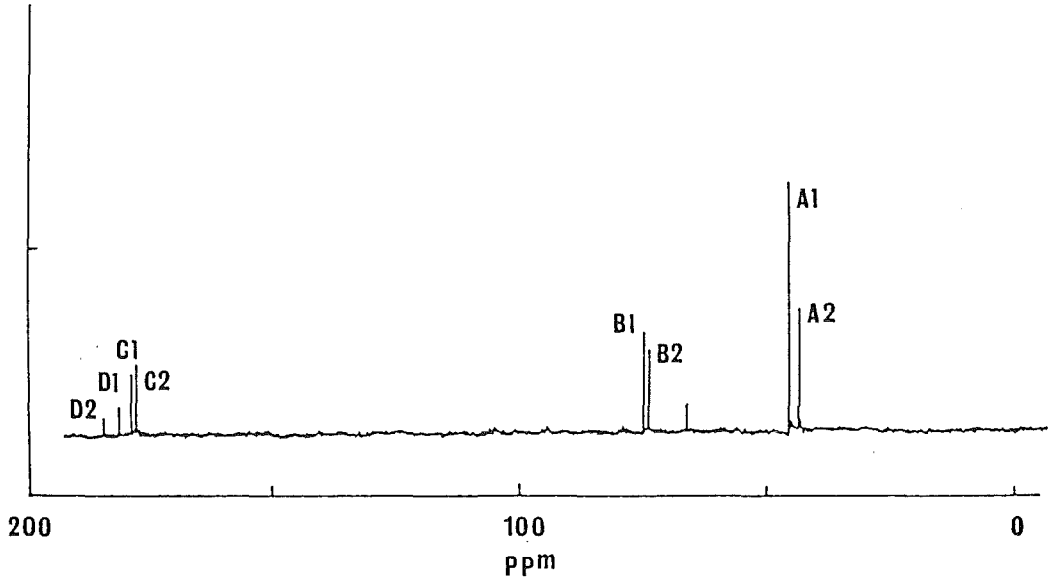


Figure 5.6 ^{13}C nmr spectrum for Al(III) (0.25 M) in citric acid (1.0 M), pH 6.0. A-D as for Figure 5.3; 1, citric acid; 2, Al-citrate complex.

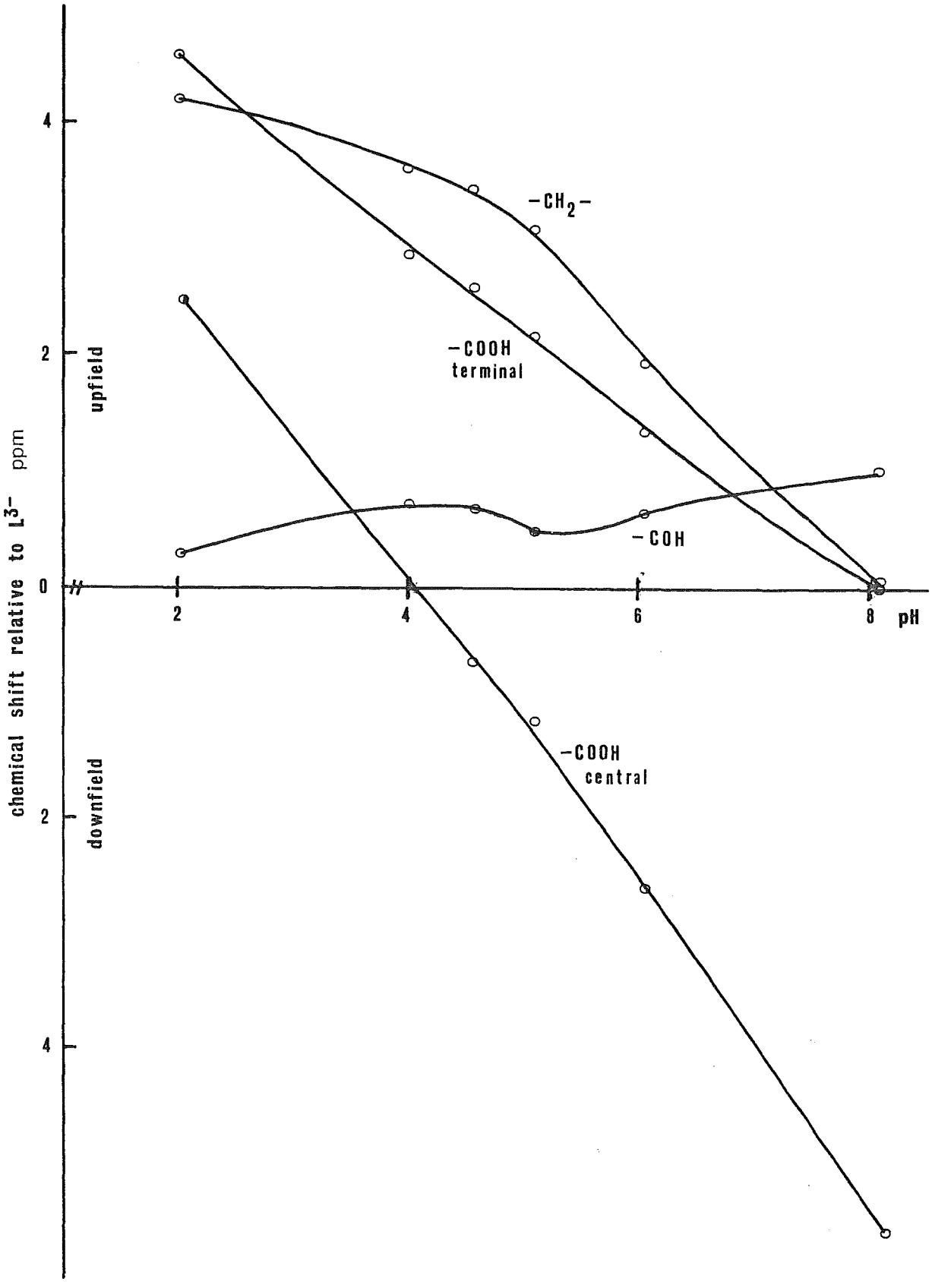
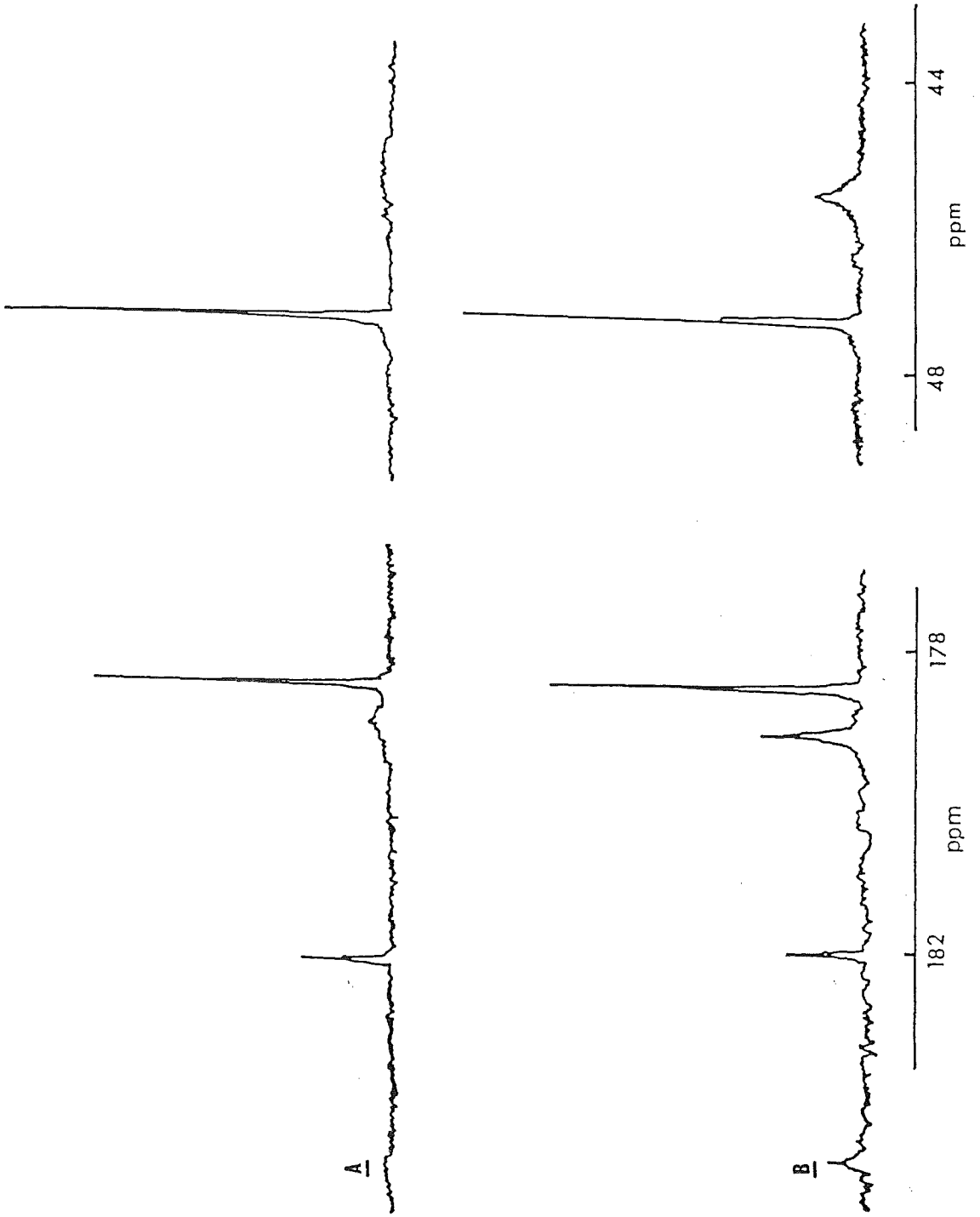


Figure 5.7 ^{13}C nmr chemical shifts for Al-citrate complexes relative to L^{3-}, as a function of pH.

Figure 5.8 ^{13}C nmr spectra (300 MHz) for Al(III) (0.25 M) in citric acid (1.0 M) at pH 4.1.
A, 23°C; B, 53°C.



a plot of chemical shifts of complexed ligand, relative to free ligand (L^{3-}), as a function of pH. Between pH 4 and 5 the -$\underline{C}$OH carbon resonance was observed to shift downfield. Above pH 5, the -$\underline{C}$OH resonance shifted upfield and the -$\underline{C}H_2$- resonance shifted substantially downfield. Both the terminal and central -$\underline{C}$OOH signals shifted downfield uniformly with increasing pH.

^{13}C nmr spectra were also recorded for aluminium - citrate solutions at 300 MHz, between pH 1.6 and 5.0. The central -$\underline{C}$OOH, terminal -$\underline{C}$OOH and -$\underline{C}H_2$- resonances were broad, and showed a temperature dependence (3 - 63^{o}C) in the pH range 3.3 - 5.0 (Figure 5.8).

5.4 DISCUSSION

5.4.1 *Protonation Constants*

Table 5.1 reports the protonation constants for the citrate(3-) ion. These are in good agreement with literature values for the same ionic strength and medium; log K_n (n=1-3) 5.72, 4.37, 2.94 (Hedwig et al., 1980). The inclusion of $K^{+}cit^{3-}$ ion pair formation lead to an increase of 0.20 in log K_1. Pearce and Creamer (1975) included ion pair formation (K=2.7; Walser, 1961) in their calculations, and obtained log K_n values of 5.83, 4.34 and 2.89 (n=1-3, 25^{o}C, I=0.10 M). In another study Still and Wikberg (1980b) used an incorrect constant for $K^{+}cit^{3-}$ formation; a value for the equilibrium constant of 3.9 mℓ meq^{-1} reported by Rechnitz and Zamochnick (1964) has been incorrectly reported as 3.9 ℓ mol^{-1} by Sillen and Martell (1971). The correct value (5.85 ℓ mol^{-1}) has been used in this work.

When solutions of low ionic strength ($I \leq 0.1$ M) are used in studying polyvalent ion reactions, significant

changes in ionic strength may occur as a result of the high charges on the ions. For example, in the titration of a triprotic acid (2.52×10^{-3} M), a change of 0.015 M in ionic strength will occur as $\bar{n}_H$ varies from 3.0 to 0.0. A discussion of the numerical approach used to correct protonation constants for variation in ionic strength is presented in Chapter 2 (Section 2.1.2). The results of such corrections are included in Table 5.1. Although no significant improvement in least squares fit was obtained, a measureable change in log K_1 (0.020 log units) was observed.

5.4.2 *^{13}C NMR Study of Citric Acid*

With increasing pH up to pH 7, the carbon resonances of citric acid are observed to shift downfield (Figure 5.4). This shift coincides with deprotonation of H_3L to form L^{3-}. The -$\underline{C}$OOH (terminal) and -$\underline{C}$OOH (central) resonances show similar pH dependences, indicating that all three carboxyl groups deprotonate simultaneously. Above pH 7 and below pH 14, the chemical shifts remain fixed, as only L^{3-} exists in solution.

Above pH 14, the resonances were again observed to shift downfield. The shift was most marked for the -$\underline{C}$OOH (central) and -$\underline{C}H_2$- carbons (Figure 5.4). Initially this shift was thought to represent deprotonation of the alkoxy group. However, when a similar study with tricarballylic acid was carried out, a similar pattern of chemical shift dependence on pH was observed. As the pH is raised above pH 14 there is a significant increase in ionic strength. When the ionic strength was held constant at high pH, by addition of LiCℓ, the pH dependence of chemical shifts for

the two acids was small and similar (Figure 5.5). The slight difference in pH dependence of the chemical shifts between the two acids gave no indication of citric acid alkoxy deprotonation.

5.4.3 *Choice of Equilibrium Model*

Interpretation of the aluminium(III) - citrate titration curve is made difficult because no distinct inflexions are observed (except at the end point) to indicate stoichiometric formation of complex species. It is also difficult to collect data at the end point, especially at low ligand : metal ratios, because of severe pH drifting. In this study, ligand : metal ratios of > 4 were used to minimize pH drift.

(a) Aluminium(III) Hydrolysis Products

In a study of metal-ligand complexing, the hydrolysis reactions of the metal ion need to be considered. Below pH 4 aluminium (III) exists as the hexa-aquo ion $Al(H_2O)_6^{3+}$, and in alkaline solutions it exists as $Al(OH)_4^-$ (Campbell et al., 1983). However, for intermediate pH the choice of hydrolysis models proposed for aluminium(III) is wide.

Schofield and Taylor (1954) and Frink and Peech (1963) postulated $AlOH^{2+}$ as the only hydrolysis product formed in dilute solutions (< 0.001 M). Aveston (1965) interpreted results from extensive ultracentrifugation and acidity measurements of hydrolysed aluminium perchlorate solutions in terms of the dimer $Al_2(OH)_2^{4+}$, and the polynuclear species $Al_{13}(OH)_{32}^{7+}$. This polynuclear species has been identified by Raman spectroscopic studies (Waters & Henty, 1977), and characterized in the solid state (Johansson, 1960).

Extensive ^{27}Al nmr studies have been performed on aluminium(III) solutions (Akitt & Farthing, 1978, 1981a-d; Akitt & Milic, 1984). Akitt and Farthing have shown that the hydrolysis products of aluminium(III) are dependent on the way the hydrolysis is carried out. Rapid hydrolysis with sodium carbonate produced solely the polynuclear cation $Al_{13}(OH)_{32}^{7+}$, whereas dissolution of aluminium metal in aqueous $AlCl_3$ resulted in a minor amount of $Al_{13}(OH)_{32}^{7+}$ and two species whose structures were unknown.

From potentiometric studies, Mesmer and Baes (1976) postulated the hydrolysis species $AlOH^{2+}$, $Al(OH)_2^+$, $Al(OH)_3$, $Al(OH)_4^-$, $Al_2(OH)_2^{4+}$, $Al_3(OH)_4^{5+}$ and $Al_{13}(OH)_{32}^{7+}$. Two schemes of hydrolysis products described the experimental data equally well, one including $Al_2(OH)_2^{4+}$ and $Al_3(OH)_4^{5+}$, and the other including $Al_2(OH)_3^{3+}$ and $Al_3(OH)_3^{6+}$. The authors preferred the $Al_2(OH)_2^{4+}$ and $Al_3(OH)_4^{5+}$ scheme because of the apparent absence of (2,3) species in other known metal hydrolysis systems.

Ohman and Forsling (1981) included three species in their model; they were $AlOH^{2+}$, $Al_3(OH)_4^{5+}$ and $Al_{13}(OH)_{32}^{7+}$. The dimer $Al_2(OH)_2^{4+}$ was considered, but resulted in larger errors in log β values.

Brown et al. (1985) identified the hydrolysis species $Al(OH)^{2+}$, $Al(OH)_2^+$, $Al_3(OH)_4^{5+}$ and a high molecular weight polymer with OH/Al $\underline{c}$ 2.46; the polynuclear species $Al_{13}(OH)_{32}^{7+}$ has this ratio. This work was carried out at 25°C (I = 0.1 M $NaNO_3$) with $[Al^{3+}] \leq 0.1$ M. The most important species was found to be $Al(OH)^{2+}$. $Al(OH)_2^+$ only became significant at high pH and at low Al^{3+} concentrations. $Al_3(OH)_4^{5+}$ was not an important hydrolysis product at this

low Al^{3+} concentration. The polynuclear species only became important at pH > 5.

The model of Ohman and Forsling (1981) was used in this study. This model includes the species that were generally considered of major importance at low Al^{3+} concentrations, viz. $Al(OH)^{2+}$, $Al_3(OH)_4^{5+}$ and $Al_{13}(OH)_{32}^{7+}$.

(b) End Point Stoichiometry

To more accurately define the end point stoichiometry, two experiments were performed. Firstly, titration of an aluminium - citrate solution with a large excess of ligand (ligand:metal 12.7:1) established that 2.0 $\pm$ 0.2 moles of protons were released per mole of aluminium. Data from this titration could not be used in a least squares analysis to determine stability constants because of the large contribution of protons from the excess ligand.

Secondly, the protons released when a citrate solution (pH 8.7) was titrated with Al^{3+} indicated that 2.2 $\pm$ 0.2 moles of protons were released per mole of aluminium. The addition of each increment of Al^{3+} lead to a decrease in pH. The proton release was estimated from the KOH titre required to maintain constant pH. Further, the decrease in pH diminished with each addition of Al^{3+}, but the same number of protons were titrated. This result indicated that a species was forming which was able to buffer the release of protons, i.e. a weak base. The concentration of weak base, and consequently the buffering capacity, will increase with each increment of Al^{3+}. Both of these experiments indicated an end member species of the form $Al(LH_{-1})_2^{5-}$, $Al(OH)_2L_2^{5-}$ or $Al(OH)(LH_{-1})^{2-}$.

(c) Non-Linear Least Squares Analysis

On the basis of previously reported models, the species $AlHL^{+}$, AlL and AlL_2^{3-} were included in a "starting" model. The model was modified by addition or subtraction of species as indicated by the sign and the magnitude of the residuals $T_H(obs) - T_H(calc)$, (see Section 2.5), and the R-factor (Vacca et al., 1972).

None of the previously reported models included a species $Al_x(H_{-2})_xL_y$, as indicated present at the end point, although Jackson (1982) included $AlL_2(OH)^{4-}$ in his model. It is conceivable that at higher pH this could form $AlL_2(OH)_2^{5-}$. A satisfactory fit to the experimental data was obtained for the equilibrium species $AlHL^{+}$, AlL, AlL_2^{3-}, $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$ (R-factor 0.29 - 0.75%). However, there was a region of poor fit between pH 3.4 and 5.1, indicating the need for a further species to be included in this region.

The inclusion of $Al(HL)L^{2-}$ improved the fit, with R-factors ranging from 0.16 to 0.31% for individual data sets (Figure 5.2). This species has been postulated for the zinc-citrate system (Christie et al., 1986).

Inclusion of the species $AlL(OH)^{-}$, as postulated by Jackson (1982) and Motekaitis and Martell (1984), also improved the fit in this region. However, this species was rejected because,

(i) $AlL(OH)^{-}$ was calculated to exist in solution at pH < 3, a pH much lower than -log β for the first hydrolysis reaction of Al^{3+}.

(ii) The pH-dependent distribution curve showed this species increasing in concentration then

decreasing, then again increasing in a region where the only other species present are bis species, i.e. $AlL(OH)^-$ must form from AlL_2 by dissociation of a coordinated ligand. At higher pH, a ligand must again coordinate to form the deprotonated bis species $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$. This coordination of L^{3-} would be occurring in a region where the concentration of L^{3-} is not increasing. The more likely pathway to the formation of the deprotonated bis species would be from AlL_2^{3-} by ligand alkoxy deprotonation.

Lopez-Quintella et al. (1984) postulated the existence of AlH_2L^{2+} in acidic solutions (pH < 2.7). Inclusion of this species in the model did not improve the fit.

(d) Alternative Models

The possibility of dimer formation was examined in an alternative model. The species most likely to form dimers is AlL. This species contains aluminium ions with available coordination sites, and ligands with an uncoordinated (terminal) carboxylate group. Reactions (5.4), (5.7), (5.8), (5.10) and (5.11)

$$2Al^{3+} + 2H_2L^- \Leftrightarrow Al_2L_2 + 4H^+ \qquad (5.10)$$

$$Al_2L_2 + 2L^{3-} \Leftrightarrow 2AlL_2^{3-} \qquad (5.11)$$

were included in the model. This model gave larger R-factors (0.37-1.29%) than that proposed, and was rejected. Further, the presence of a dimer would be indicated by a concentration

dependence of the stability constant associated with the monomer. No evidence of total metal or total ligand concentration dependence was found when the constants were compared for two different absolute concentrations of Al^{3+} and citrate at $T_L/T_M = 6.34$.

Polynuclear citrate complexes have been proposed for several metal ions, e.g. $Al_3(OH)_4L_3^{4-}$ (Ohman & Sjoberg, 1983), $Ni_4(OH)(LH_{-1})_3^{5-}$ (Still & Wikberg, 1980a) and $Cu_2(LH_{-1})_2^{4-}$ (Still & Wikberg, 1980b). The composition of these polynuclear complexes relates to the dominant metal ion hydrolysis species, i.e. $Al_3(OH)_4^{5+}$, $Ni_4(OH)_4^{4-}$ and $Cu_2(OH)_2^{2+}$. However, in this work, no evidence was found to support such an aluminium - citrate species.

The models proposed by Jackson (1982), Ohman and Sjoberg (1983), and Motekaitis and Martell (1984) were considered. The model of Motekaitis and Martell (1984) could be refined using the data sets of this work. The computed constants (log K $AlHL^+$, 10.87; AlL, 8.02; AlL <—> $Al(OH)L^-$ + H^+, -3.60) were in good agreement with those reported (10.92, 7.98, -3.31), indicating that the data sets of the two studies were equivalent. However, the model was not considered correct because the fit was poorer (R factor $\underline{c}$ 0.7%) than the model proposed in this study, and the end point stoichiometry was not consistent with that determined in this study.

Neither the Ohman and Sjoberg (1983) model nor Jackson's (1982) model could be refined with the data sets obtained in this work. In each case, one of the complexes was mathematically rejected. With the exclusion of these parameters ($Al_3(OH)_4L_3^{4-}$ and $Al(OH)L_2^{4-}$ respectively), the two models reduced to that proposed by Motekaitis and Martell (1984).

5.4.4 *The Equilibrium Model*

The model which gave the best non-linear least squares fit to the experimental data included the ligand species H_3L, H_2L^-, HL^{2-} and L^{3-}, the aluminium hydrolysis species Al^{3+}, $AlOH^{2+}$, $Al_3(OH)_4^{5+}$ and $Al_{13}(OH)_{32}^{7+}$, and the complexes $AlHL^+$, AlL, $Al(HL)L^{2-}$, AlL_2^{3-}, $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$. The computed distribution curves for this model are shown in Figure 5.2.

A good model must not only give a good numerical fit to experimental data, but it must also be based on sound chemical sense. There must be a sensible route for formation and replacement of every complex proposed.

(a) $Al(HL)^+$

In $Al(HL)^+$ the ligand is considered to be tridentate. This follows from comparison of log K ($Al^{3+} + HL^{2-} \Leftrightarrow Al(HL)^+$) for citrate (5.07) with that for bidentate tricarballylate (3.14; Sillen & Martell, 1971). It is also consistent with the values for tridentate (AlL) tricarballylate (5.44; Jackson, 1982) or malate (5.34; Sillen & Martell, 1971).

(b) AlL

The constant for the formation of AlL (log K 8.35) is considerably larger than those for other tridentate ligands with aluminium (tricarballylic acid, 5.44 and malate, 5.34; Jackson, 1982).

Motekaitis and Martell (1984) postulated that this additional stability was due to coordination of the alkoxide group ($-O^-$). This would require that formation of the deprotonated bis complexes, $AlL(LH_{-1})^{4-}$ and $Al(LH_{-1})_2^{5-}$,

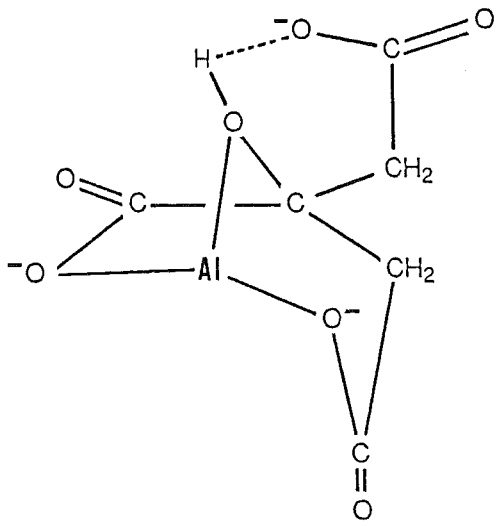


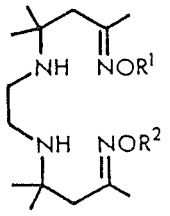
Figure 5.9 Proposed structure of [AlL], indicating mode of coordination and intramolecular hydrogen bonding (coordinated waters omitted).

be formed from AlL_2 by carboxyl group deprotonation. However, this seems most unlikely as the constants for these reactions are much greater than that for the equivalent deprotonation reaction for the free ligand, i.e. $-\log K\ (HL^{2-} \Leftrightarrow H^+ + L^{3-}) = -4.35$.

As HL in $Al(HL)^+$ is proposed to be tridentate, and as it is not possible for the ligand to be tetradentate, AlL must also be tridentate. One can interpret the additional stability of the AlL complex as resulting from intramolecular hydrogen bonding if coordination of the ligand is through two carboxylate groups and the alkoxy group (—OH). In the case of $Al(HL)^+$, the free carboxyl group is still protonated. However, to form AlL, this carboxyl group deprotonates. Strong intramolecular hydrogen bonding between the non-coordinated carboxylate group and the coordinated alkoxy group can occur. A terminal carboxylate group would be in a more favourable position to hydrogen bond than would be the central carboxyl group. Hydrogen bonding of the terminal group would give the most favourable combination of ring sizes in the complex (Figure 5.9).

Baker et al. (1983) reported the structure of a nickel - citrate complex $[Ni_2(H_2O)_4L_2]^{4-}$, in which an intracitrate hydrogen bond -OH --- $^-$OOC- is clearly indicated.

The stability resulting from intramolecular hydrogen bonding has been reported by Daniel et al. (1978) for deprotonation of the oxime - copper (II) complexes (A) and (B),



R^1	R^2	
H	H	(A)
H	Me	(B)

Whereas the oximate group in (B) can only hydrogen bond with the solvent, that formed by (A) can bond intramolecularly, and the overall stability of the complex ($M^{2+} + L \Leftrightarrow MLH_{-1} + H^+$) is increased by 3.5 log units.

For the above argument to be invoked to explain the high stability of AlL, intramolecular hydrogen bonding must not contribute to the stability of the free ligand. Although pK_3 for citric acid is 0.59 log units lower than that for tricarballylic acid (Jackson, 1982), comparison of the values for acetic acid (4.7) and glycollic acid (3.6) suggest that the difference is the result of an electron withdrawing affect of the alkoxy group on the central carbon atom. In contrast to the relatively free orientation of the alkoxy group in the citrate ion, its fixed geometry in the complex may facilitate intramolecular hydrogen bonding.

(c) $Al(HL)L^{2-}$

The complex $Al(HL)L^{2-}$ forms between AlL and AlL_2^{3-} (Figure 5.2), and gives a plausible route to formation of AlL_2^{3-} from AlL. $Al(HL)L^{2-}$ could form either from

$AlHL^{+} + L^{3-}$, or from $AlL + HL^{2-}$. The high concentration of HL^{2-} in solution when AlL is forming would tend to favour the latter route of formation.

(d) AlL_2^{3-}

This bis complex could form by deprotonation of $Al(HL)L^{2-}$ or by addition of L^{3-} to AlL. AlL_2^{3-} is present over a wide pH range (3 - 7) and is formed in a region where L^{3-} is present in solution.

Formation of a bis species is consistent with formation of a bis-malate species (Perrin, 1979) and a bis-malonate species (Sillen & Martell, 1971).

The significantly larger constant for the formation of the bis complex, compared with other tridentate bis complexes (e.g. malate, 3.98; Sillen & Martell, 1971), may also be attributed to hydrogen bonding within the complex.

(e) $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$

The presence of a complex of the form $Al_x(H_{-2})_xL_y$ was indicated earlier (see Section 5.4.3b). The site of deprotonation of the bis complex, to form $AlL(LH_{-1})^{4-}$ and $Al(LH_{-1})_2^{5-}$, is most likely from the coordinated alkoxy group of the ligand. Alternatively, proton loss to form $AlL_2(OH)^{4-}$ and $AlL_2(OH)_2^{5-}$ would require dissociation and replacement of a coordinated ligand group with an OH group. Deprotonation of the bis species is supported by ^{13}C nmr results (see Section 5.4.6).

5.4.5 *Analysis of Variance*

It is usual to perform several titrations on an equilibrium system to establish the reproducibility of a titration. Protonation or stability constants may be determined either for a combined data set, or for each data set separately followed by averaging constants.

Braibanti et al. (1982) applied statistical analysis of variance to the glycinate-nickel system to determine whether data could be processed as a whole, or whether data sets should be processed separately.

If all data points (i) of m titrations belong to the same population, then it is possible to calculate an average constant (log β) by refining the combined data set. To establish whether all data points belong to the same population, each data set (k) is refined separately, and values of log β(k) are calculated with variance σ^2(k). A grand average, log β, with variance σ_{av}^2 is calculated from all log β(k) values. The null hypothesis (H_o) that all data belong to the same population is accepted if $F = (n\sigma_{av}^2/\sigma_i^2) \simeq 1$, where $\sigma_i^2 = \Sigma\sigma^2(k)/m$ and n is the number of data points contributing to the constant. If the null hypothesis holds, then it is established that all data points belong to the same population, and the combined data set can be used. If H_o is rejected, then refinement must be done separately for each titration.

In practice, it is generally observed that the variance between data sets is greater than the variance within data sets. Therefore, in this work the data sets were processed separately, and a grand average for each constant calculated from the values derived for each titration.

5.4.6 *^{13}C NMR Studies*

The ^{13}C nmr spectra of aluminium - citrate solutions, between pH 2 and 8, showed four pairs of resonance lines (Figure 5.6). Each pair of resonance lines is associated with one of the four carbon environments, one line corresponding to the free ligand and the other to the complex. The lines were assigned by comparison with ligand-only spectra at the same pH.

The existence of pairs of resonance lines indicated that ligand exchange was slow on the time scale of the nmr experiment. Only one resonance line was observed for the terminal COOH groups in the complex, even though one group is coordinated and the other probably uncoordinated (see Section 5.4.4b). Thus, there must be rapid exchange between these groups, resulting in an averaged signal. The presence of eight resonance lines at pH 2 also indicated that complexing commenced below pH 2.

The resonance frequencies for complexed citrate are subject to two factors, (i) the effect of ligand deprotonation, shifting the resonances downfield as for free ligand (see Figure 5.4), and (ii) the effect of changes in carbon environments arising from metal ion coordination or changes in conformation or composition of complexes.

The effect of ligand deprotonation can be corrected for by measuring the signal for complexed ligand relative to uncomplexed ligand in the same state of protonation, e.g. $AlHL^{+}$ measured relative to HL^{2-}, and AlL measured relative to L^{3-}.

It would be incorrect to measure the signal for complexed ligand relative to that for uncomplexed ligand at

the same pH because composition of the ligand protonation states would differ. For example, at pH 3.5, the composition of the ligand-only solution would be 16% H_3L, 73% H_2L^- and 12% HL^{2-} (Figure 5.1), whereas the aluminium(III) - citrate solution would contain 8% $AlHL^+$, 70% AlL, 15% $Al(HL)L^{2-}$ and 8% AlL_2^{3-} (Figure 5.2).

Without prior knowledge of the exact solution composition at any given pH, it would not be possible to determine the combination of L^{3-} and HL^{2-} required. Further, it is not possible to determine the chemical shift for solely HL^{2-} because it always exists in solution in equilibrium with significant amounts of H_2L^- and L^{3-}.

The only protonated states of ligand which exist alone in solution and can therefore be assigned chemical shifts are H_3L and L^{3-}. H_3L does not form complexes with Al(III). Consequently, complexed ligand resonances were measured relative to L^{3-}.

Changes in relative chemical shifts (δ(complexed ligand) - $\delta(L^{3-})$) with changes in pH may result from

(i) deprotonation of coordinated ligand, e.g.

$AlHL^+ \longrightarrow AlL$,

$Al(HL)L^{2-} \longrightarrow AlL_2^{3-}$,

$AlL_2^{3-} \longrightarrow AlL_2H_{-1}^{4-}$,

$AlL_2H_{-1}^{4-} \longrightarrow Al(LH_{-1})_2^{5-}$,

(ii) a change in carbon environment arising from a change in conformation, or composition,

or

(iii) a change in carbon environment due to metal ion coordination. Coordination of Al(III) will

result in a deshielding effect on neighbouring carbon atoms as it withdraws electron density from them, shifting the resonances downfield relative to free ligand.

The central -COOH and terminal -COOH resonances for complexed ligand showed continuous downfield shifts over the pH range 2 to 8, relative to the resonance for L^{3-}. This downfield shift may be the result of Al(III) coordination. Above pH 5, the downfield shift may also correspond to deprotonation of the alkoxy group to form $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$.

The methylene carbon resonance was observed to shift significantly downfield with increasing pH. Further, above pH 5 there was a change in slope, the resonance shifting more markedly downfield over a narrower pH range. The shift may have been due to Al(III) coordination. However, the methylene carbons are furthermost from the metal centre and therefore least likely to be affected by metal ion coordination. The change in slope occurred over the pH range where deprotonation of the bis complexes is postulated, i.e. alkoxy group deprotonation.

The central carbon resonance (- COH) was shifted least with increasing pH. Very little change was observed below pH 4, suggesting that this carbon is not significantly affected by metal ion coordination, or that the effect of metal ion coordination is counteracted by some other effect. A slight downfield change followed by a gradual upfield change was observed above pH 5. An upfield shift is not consistent with ligand deprotonation (to form $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$) or metal ion coordination.

However, the shift may result from a conformational change as the alkoxy group deprotonates, e.g. loss of hydrogen bonding.

Strouse (1977) has reported ^{13}C nmr chemical shift patterns for Fe(II)-citrate solutions. A large upfield shift was observed for the central carboxyl carbon resonance. The methylene carbon and terminal carboxyl carbon signals showed large downfield shifts.

The Fe(II)-citrate ^{13}C nmr results were consistent with those reported for Ni(II)-amino acid solutions (Strouse and Matwiyoff, 1970), where the hydroxyl oxygen of citrate replaces the nitrogen of the amino acid.

The chemical shift patterns observed in this work for Al(III)-citrate solutions were similar to those observed for the Fe(II)-citrate system and the Ni(II)-amino acid system. The terminal carboxyl carbon and methylene carbon resonances were shifted substantially downfield, and the central carbon resonance moved upfield as the pH was raised.

A second series of ^{13}C nmr spectra were recorded for aluminium - citrate solutions between pH 1.6 and 5.0 but at 300 MHz. The signals for the carboxyl and methylene groups were broad and structured throughout the pH range, indicating the presence of more than one chemically distinct environment for each carbon atom. The broad nature was present even in solutions where > 80% of one complex species was present (e.g. pH 5). Between pH 3.3 and 5.0, the resonances sharpened with increasing temperature (3 - 63^{o}C, Figure 5.8). This temperature dependence (a coalescence of resonances) is indicative of increasing rate of exchange

between coordinated ligand species. Specifically, the broad nature of the resonance lines could arise from

(i) the presence of both coordinated and uncoordinated terminal carboxyl groups in the one complex,

(ii) equilibrium between coordination isomers,

or

(iii) the presence of mono and bis complexes.

Although the resonances sharpened with increasing temperature, they did not shift relative to the peak for uncomplexed ligand. This indicates that ligand exchange in the mono complexes (e.g. AlL and $Al(HL)^{+}$) must be slow on the nmr time scale. If ligand exchange were rapid enough, then an average signal of free ligand and complexed ligand would be seen at an intermediate frequency.

In support of the proposed model for the aluminium(III) - citrate equilibria, four points can be made :

(i) The model gave the best non-linear least squares fit to the experimental data.

(ii) All of the proposed species were formed from, and replaced by, sensible species, i.e. the model made chemical sense.

(iii) The model included a complex $Al_x(H_{-2})_xL_y$ consistent with the end point stoichiometry.

(iv) The pH regions where $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$ were proposed, were supported by regions of change in ^{13}C nmr spectra.

CHAPTER 6

ACID PYROPHOSPHATE EXTRACTION OF SOIL FULVIC ACIDS

6.1 INTRODUCTION

The first task preceding any research on soil humic substances is to isolate the organic matter from the soil matrix. Methods for isolation of soil organic matter frequently involve an initial extraction at pH > 13. This is followed by acidification to separate fulvic acids from acid insoluble humic acids. In both steps oxidation of the organic matter may occur. The products isolated may be artifacts of the original soil components.

Commonly, the fulvic acid component of humic substances is recovered by acidification of an alkaline solution containing both humic and fulvic acids. However, because of its solubility, it is possible to extract fulvic acid using acidic conditions with minimal release of humic acid.

The aim of this work was to develop an extraction procedure for soil fulvic acid using acidified pyrophosphate solution. Conditions were sought for the release of minimal quantities of humic acid, and minimal alteration of the organic matter. A variety of resins were studied for their effectiveness in separating the fulvic acid from the pyrophosphate extractant.

6.1.1 *Criteria for Extraction Methods*

Stevenson (1982) has listed four objectives that should be considered when developing an extraction process for humic substances :

- complete extraction to ensure a representative sample
- extraction of material in an unaltered form
- minimal extraction of inorganic components
- a method applicable to all soils.

To avoid the formation of artifacts during the extraction of fulvic acids, mild conditions (neither strongly acidic or alkaline) should be used. The extractant solution should be at pH < 7 to avoid oxidation of phenolic components or hydrolysis of ester linkages, and to minimize the release of humic acids. A reagent capable of sequestering ferric ions must be present, if extracting under acidic conditions, to block possible redox oxidation reactions of phenolics, i.e.

$$2Fe^{3+} + \text{phenol} \Leftrightarrow 2Fe^{2+} + \text{quinone} + 2H^{+}$$

In an extraction process three steps are required; removal of the organic matter from the soil matrix, separation of organic matter from the extractant, and purification. In all three steps care must be taken to prevent the loss of material. Distinct fractions may be lost during each step due to their specific chemical or physical properties.

If quantities of inorganic material are co-extracted with the organic matter then they must be removed, either during the separtion step (separation of the organic matter from the extractant) or by a purification step. Fulvic acids with low ash contents are required for characterization

of organic matter and for studies of proton and metal binding.

Various extraction methods were discussed in Chapter 1. (Section 1.5.1). One of the methods described involved extraction with acidic pyrophosphate solution and isolation on XAD-7 resin (Gregor and Powell, 1986a). This extraction procedure will be discussed in some detail in this chapter. Specifically, the emphasis will be placed on the properties of XAD resins that lead to their choice for the separation of fulvic acid from the extractant.

6.2 THE EXTRACTANT METHOD

SEE ERRATA

This section describes the use of acidic pyrophosphate to selectively release fulvic acids from soils, with their subsequent separation from the extractant on XAD-7 resin.

The method (Scheme 6.1) has been applied to four soils, including the International Humic Substances Society reference peat sample.

Typically, 300 g of soil (200 g of peat) required 800-1000 mℓ (1600 mℓ) of pyrophosphate solution (0.1 M, pH 2) to release the fulvic acids, step (i); elution rate 2 mℓ m^{-1} (Figure 6.1a). The pH of the leachate was increased to 3-3.5 by the soil or 4-4.5 by peat, indicating that protons were being consumed during the leaching process. Some mineral matter and a small amount of humic acid were also removed from the soil during leaching. The eluate was concentrated to c 200 mℓ, by rotary evaporation, and stored at 4°C for three days, step (ii). The pH was lowered to 1.7 to precipitate humic acids, step (iii), which were removed by centrifugation. After no more than 18 h at this pH, the acidified solution was concentrated to c 100 mℓ and

filtered (finally) through a 0.025 μ membrane, step (iv).

The fulvic acids were separated from the extractant by adsorption on to XAD-7 resin (Figure 6.1.b). Three columns (10 x 300 mm, 10 x 200 mm, 10 x 200 mm) of XAD-7 were pretreated with HCℓ, pH 1.7. The fulvic acid was adsorbed onto two sequential columns, step (v), then the columns eluted with HCℓ (pH 1.7) to remove residual extractant from the resin void. Finally the columns were eluted with distilled water to remove chloride ions, step (vi). The organic matter released by the HCℓ and distilled water washings (due to a decrease in ionic strength) was recovered on a third column of XAD-7. The washings were concentrated to c 30 mℓ, eluted through the third XAD-7 column then the column washed with HCℓ and distilled water. A total of c 95% of the organic matter remained adsorbed on the three resin columns, as determined by visible absorption at 465 nm and ultraviolet absorption at 300 nm.

Recovery of the fulvic acids from the resin, step (vii), was achieved by raising the pH to and maintaining it at pH 6.5, thus ionizing the acidic functional groups. A batchwise treatment was used to control the pH, thus allowed a nitrogen atmosphere to be maintained (Figure 6.1c). Four batch treatments were required to release 98% of the adsorbed fulvic acids. A further 1% was released at pH 11.0.

Fulvic acid was converted to its acid form by cation exchange on Dowen 50W-8X resin (3 columns), step (viii). For the 1% organic matter recovered at pH 11.0, cation exchange was carried out under a (closed) N_2 atmosphere. Finally, the fulvic acid solution was concentrated to c 20 mℓ, filtered through a 0.025 μ membrane and freeze dried, step (ix) (Figure 6.1d).

SCHEME 6.1 EXTRACTION OF SOIL FULVIC ACIDS

(i) LEACHING

0.1 M $Na_2H_2P_2O_7$, pH 2

↓

(ii) Stand at $4^{\circ}C$ 3 days

↓

Concentrate and centrifuge

↓

(iii) ACIDIFICATION, pH 1.7

↓

(iv) Centrifuge, concentrate, filter (0.025 μ)

↓

(v) ADSORPTION ON XAD-7

↓

(vi) Wash to remove $H_2P_2O_7^{2-}$

(a) 0.02 M HCℓ

(b) distilled water

↓

(vii) DESORPTION pH. 6.5

↓

(viii) CATION EXCHANGE

↓

Concentrate; filter (0.025 μ)

↓

(ix) FREEZE DRY

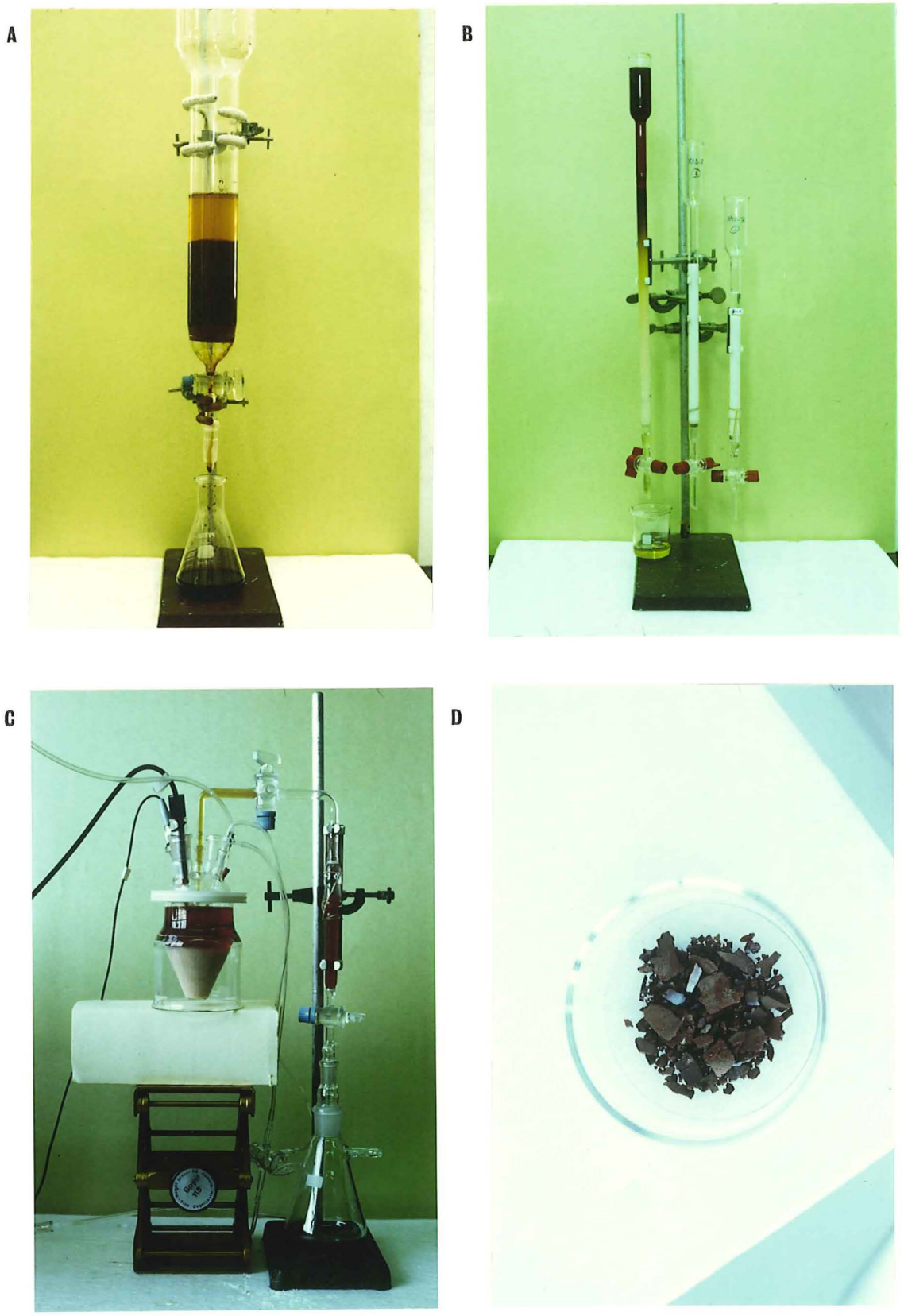


Figure 6.1 Acid pyrophosphate/XAD-7 extraction of soil fulvic acids. A, soil leaching; B, adsorption of fulvic acid on XAD-7 resin; C, desorption of fuvlic acid from XAD-7 resin; D, freeze dried fulvic acid.

Fulvic acids were extracted from four soils using this method. Two samples were from B_h horizons of a podzol soil, one from a B_s horizon of a podzol soil, and one from the IHSS peat. Their ash compositions and elemental analyses (C, H, N, O) are given in Tables 7.1 and 7.2 respectively.

6.3 CHOICE OF EXTRACTANT

The choice of extractant represents work carried out prior to this study (Gregor, 1983), and will only be briefly outlined here.

A suitable extractant for fulvic acids from soils must be capable of strongly complexing those metal ions that bind it to soil particles, specifically Fe^{3+} and Al^{3+}. Pyrophosphate meets this requirement. It forms stable soluble complexes with Fe^{3+} and Al^{3+} at low pH. Another advantage of acid pyrophosphate ($H_2P_2O_7^{2-}$) is that its salts with divalent ions such as Ca^{2+} and Mg^{2+} are sparingly soluble (Vogel, 1975). In other extraction procedures Ca^{2+} is sometimes removed by an initial acid leaching (Goh and Reid, 1975).

The pH of the extractant was chosen to :

(i) Avoid (potential) alkaline oxidation and hydrolysis.

(ii) Minimize the amount of humic acid and maximize the amount of fulvic acid extracted.

(iii) Minimize the inorganic content of the soil extract.

(iv) Ensure that the ferric-phenolic redox reaction would be blocked.

Pyrophosphate at pH 2.0 (0.1 M) was used as the leachate. This was buffered to pH 3-4 by the soil during leaching. Over this pH range the amount of Fe^{3+} and Al^{3+} released was minimal, yet the extraction of fulvic acid was complete. In a typical extraction 93% of the extractable organic matter was released by 800 mℓ of acid pyrophosphate. At this pH, humic acid extraction is minimal. For B_h horizons of a gley-podzol soil, this represented 30-50% of the organic matter extractable with 0.1 M NaOH.

The ability of pyrophosphate to mask the redox oxidation of phenolic components by ferric ions was established by sampled dc polarography. On incremental addition of catechol (1-5 x 10^{-4} M) to a solution of 2 x 10^{-4} M Fe^{3+} in 0.1 M pyrophosphate (at pH 2.0, 2.5, 4.0 or 5.3) no anodic ferrous wave developed and the peak height of the cathodic ferric wave was not diminished. Powell and Taylor (1982) have established that in the absence of pyrophosphate a redox reaction occurs. Thus, 0.1 M pyrophosphate was capable of blocking the oxidation reaction, at least to a pH as low as 2.0.

Leaching was observed to give a more efficient extraction than did a batch process. Further, complete extraction by leaching was indicated by a loss of eluant colour, and an extract containing minimal suspended matter was obtained.

6.4 THE SEPARATION STEP

To separate the extractant from the organic matter, a resin must be capable of selectively retarding the passage of either the extractant or the organic matter until the other

has completely eluted. A variety of resins and gels were studied for their effectiveness in separating pyrophosphate from fulvic acids.

6.4.1 *PGM 2000 Gel*

Polyethylene Glycol Dimethylacrylate gel (PGM 2000) has previously been applied to the separation of polyethylene glycols and proteins (Randau et al., 1971), dihydroxybenzenes (Seki, 1975) and plant phenolic (Goldstein, 1976).

A solution of fulvic acid in pyrophosphate (0.01 M) at pH 6.5 was passed through a column of PGM 2000 gel. The eluate was monitored for fulvic acid (by visible absorbance at 465 nm) and pyrophosphate (by ascorbic acid - molybdenum blue method following persulphate digestion).

At pH 6.5 the gel quantitatively rejected fulvic acids and adsorbed pyrophosphate. However, the gel was of little practical use in an extraction process because it could not be regenerated, i.e. the pyrophosphate could not be removed.

6.4.2 *DEAE Sephadex Gel*

The use of an anion exchange gel, diethylamino-ethyl (DEAE) Sephadex was considered. A synthetic solution containing salicylic acid (5×10^{-4} M), phthalic acid (1×10^{-3} M) and pyrophosphate (0.01 M) at pH 2 was eluted through a column of the gel. At pH 2, both salicylic and phthalic acids are predominantly monoanionic and the pyrophosphate is predominantly dianionic ($H_2P_2O_7^{2-}$). The resin has a greater affinity for dianionic species. The majority (95%) of the organic acids were eluted from the column (as detected by UV absorbance at 300 nm for salicylic acid, and 275 nm for phthalic acid) and only minor amounts

(2%) of pyrophosphate were released. However, the gel proved unsatisfactory when applied to fulvic acid/pyrophosphate solutions because the fulvic acids were strongly adsorbed.

6.4.3 *XAD Resins*

XAD resins are nonionic macroporous copolymers with large surface areas. Two types of XAD resins are available, the styrene divinylbenzene resins (e.g. XAD-1, XAD-2, XAD-4) and the acrylic ester resins (e.g. XAD-7, XAD-8). They differ in pore size (5-25 nm), surface area and polarity, and hence capacity. Hydrophobic interactions control the adsorption and desorption properties of these resins. Organic matter is adsorbed at low pH when weak acids are protonated (non-ionic), and desorbed at higher pH as the acids are ionized.

The resins have been extensively used for recovery of organic compounds from natural waters (Aiken et al., 1979; Weber and Wilson, 1975; Mantoura and Riley, 1975; Thurman and Malcolm, 1981). Little work has focussed on the use of these resins in the isolation of fulvic acids from soils. However, several factors point to their possible use :

(i) The low pH (< 2) required for adsorption of nonionic fulvic acids on XAD resins is compatable with the step to remove humic acids in any extraction process.

(ii) The ionic extractant and ions released with the fulvic acids (e.g. Fe^{3+}, Al^{3+}, Ca^{2+}) are rejected by the resin.

(iii) Acidic solutions containing the soil extract will have a comparatively high ionic strength;

uptake of organics on XAD resins is enhanced with increasing ionic strength (Gustafson et al., 1968).

Adsorption and desorption efficiencies of various XAD resins (XAD-2, XAD-4, XAD-7, XAD-8) for fulvic acid and pyrophosphate were determined in a previous study (Gregor, 1983). A brief outline of results follows :

(i) Adsorption of fulvic acid was favoured at low pH (pH 2) and high ionic strength (0.1 M pyrophosphate). The acrylic ester resins XAD-7 and XAD-8 were found to have greater capacities than the styrene divinylbenzene resins for fulvic acid (Figure 6.2).

(ii) At pH 2, the pyrophosphate extractant was found to be almost completely rejected. Residual extractant in the resin voids was adequately removed by elution with 0.02 M HCℓ.

(iii) Recovery of fulvic acid from the resins was achieved by raising the pH to 6.5, ionizing the acidic functional groups. Desorption was incomplete and slow for the styrene divinylbenzene resins. Although incomplete, desorption of fulvic acid from XAD-7 was more rapid.

XAD-8 has been chosen for use in the International Humic Substance Society programme for isolation of reference fulvic substances (Thurman and Malcolm, 1981). In this work XAD-7 was chosen because of its higher capacity and affinity for fulvic acids, as demonstrated in breakthrough curves

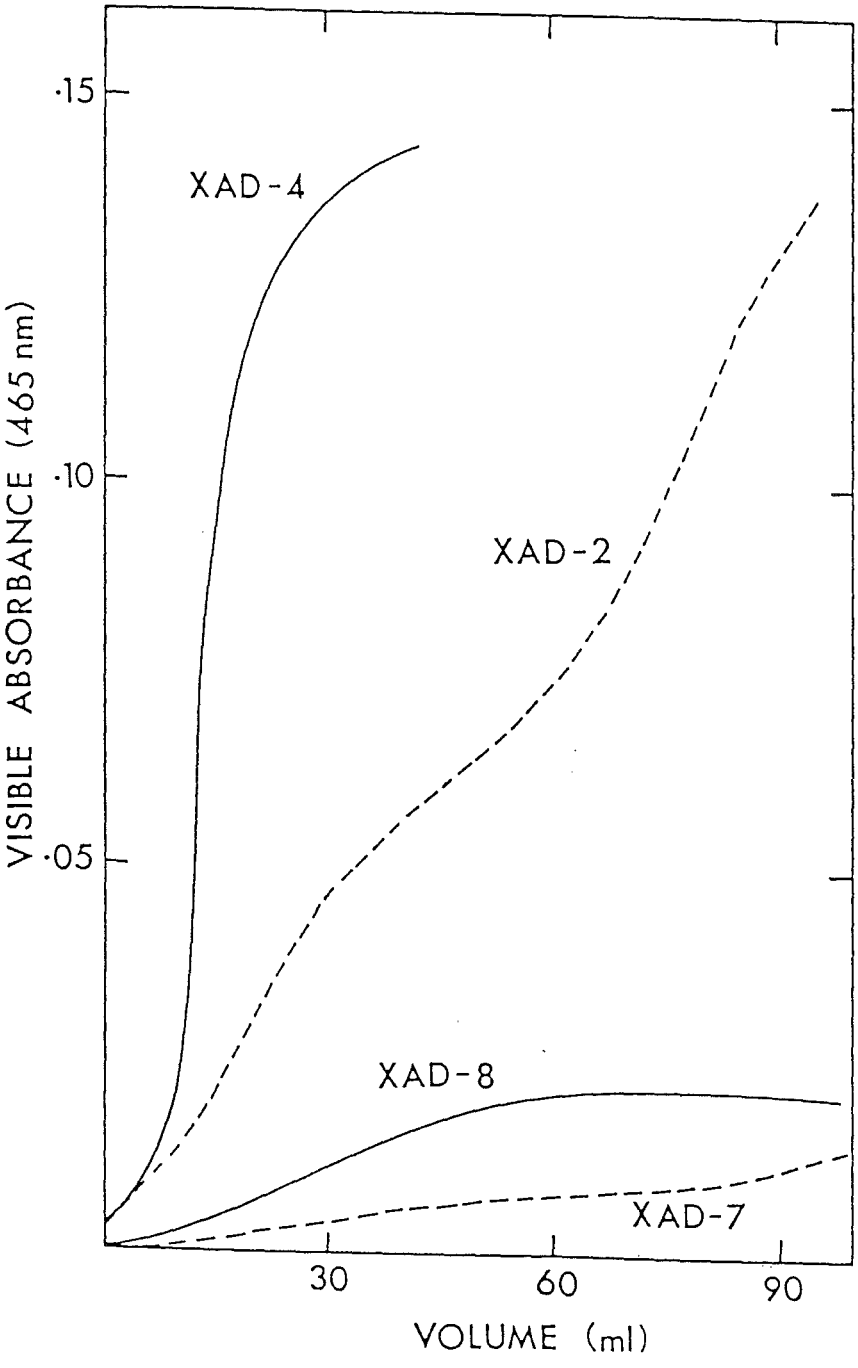


Figure 6.2 Breakthrough curves for fulvic acid solutions (200 $mg\ell^{-1}$, pH 2.0) on XAD resins (10 mℓ resin, column diameter 13 mm).

(Figure 6.2) and observed in the smaller volume of resin required to adsorb fulvic acids from a given solution. XAD-7 also showed a greater physical stability; stirring of XAD-8 resin with water at pH 6.5 during desorption was found to produce excessive fines.

6.5 ADSORPTION AND DESORPTION PROPERTIES OF XAD-7

The properties of several model compounds (for fulvic acid), and of fulvic acid itself, on XAD-7 were studied.

6.5.1 *Recovery of Phenols*

As mentioned in the previous section, incomplete recovery of fulvic acids from XAD-7 was achieved at pH 6.5. Fulvic acids contain not only polycarboxylic acids, but may also contain polymeric phenols. At pH 6.5 the majority of carboxylic acids will be deprotonated and should be released from XAD-7. However, phenolic components would remain protonated at this pH.

Desorption of the phenols, catechol, epicatechin and an epicatechin polymer, from XAD-7 was studied. The phenols (3.3, 2.1 and 0.15 x 10^{-3} M respectively) were adsorbed from 40 mℓ of solution at pH 2 onto 5 mℓ of resin. A plot of % desorption against pH generated a sigmodal curve indicating 50% desorption at pH $\simeq$ pK_1 for the phenol ($\underline{c}$ 9.6) and 100% desorption at pH > 10.5 (Figure 6.3). Therefore, to quantitatively recover these molecules with weakly acidic functional groups from XAD-7, it is necessary to desorb at a much higher pH ($\geq$ 10.5).

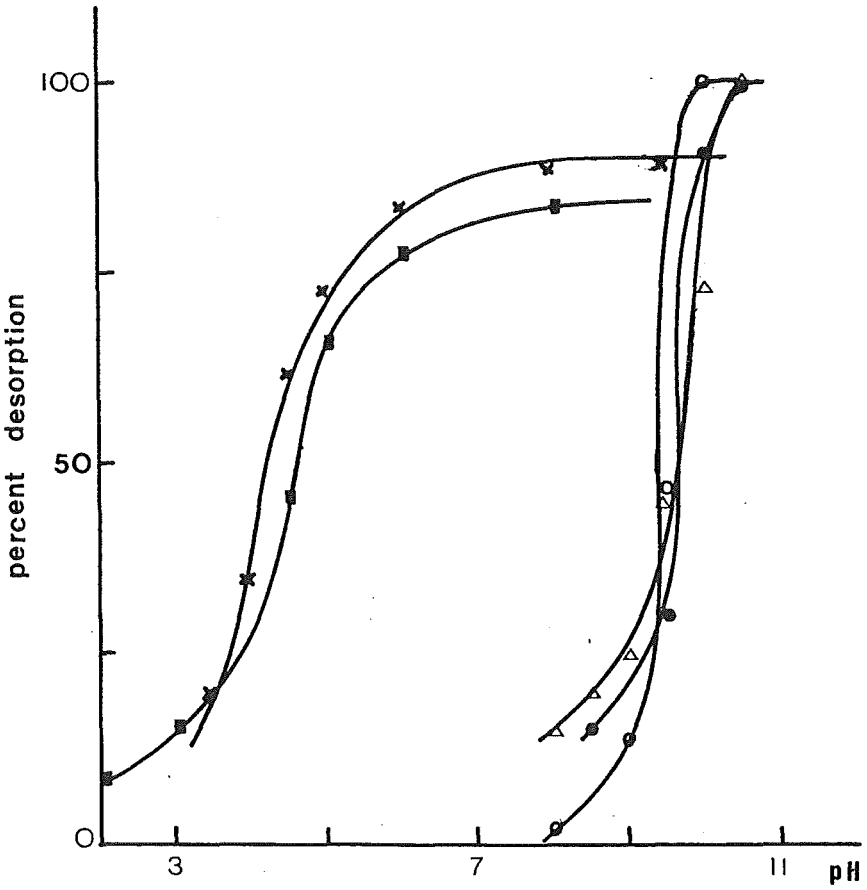


Figure 6.3 pH-desorption curves on XAD-7. Phthalic acid (x), resin loading at pH2 4.97 mg $m\ell^{-1}$; fulvic acid (■), FA1 2.03 mg $m\ell^{-1}$; catechol (Δ), 2.87 mg $m\ell^{-1}$; epicatechin (O), 4.71 mg $m\ell^{-1}$; epicatechin polymer (●), 4.41 mg $m\ell^{-1}$.

6.5.2 *Behaviour of Phthalic Acid on XAD-7*

Phthalic acid solution (0.037 M) was added incrementally to 7 g (dry weight) of XAD-7 resin in 75 mℓ of solution at pH 1.7. The concentration of phthalic acid remaining in solution following each addition was determined by monitoring the ultraviolet absorbance at 274 nm.

Desorption as a function of pH showed a sigmoidal curve (Figure 6.3), and indicated 50% desorption at pH $\underline{c}$ 4. Complete desorption was not achieved; above pH 6.5 $\underline{c}$ 17% remained adsorbed. However, following repeated removal of solution and replacement by distilled water (i.e. dilution of phthalic acid concentration in solution), complete recovery was obtained. An equilibrium between the phthalic acid in solution and the phthalic acid adsorbed on the resin was controlling desorption. Further phthalic acid could only be released from the resin when the concentration in the solution was lowered.

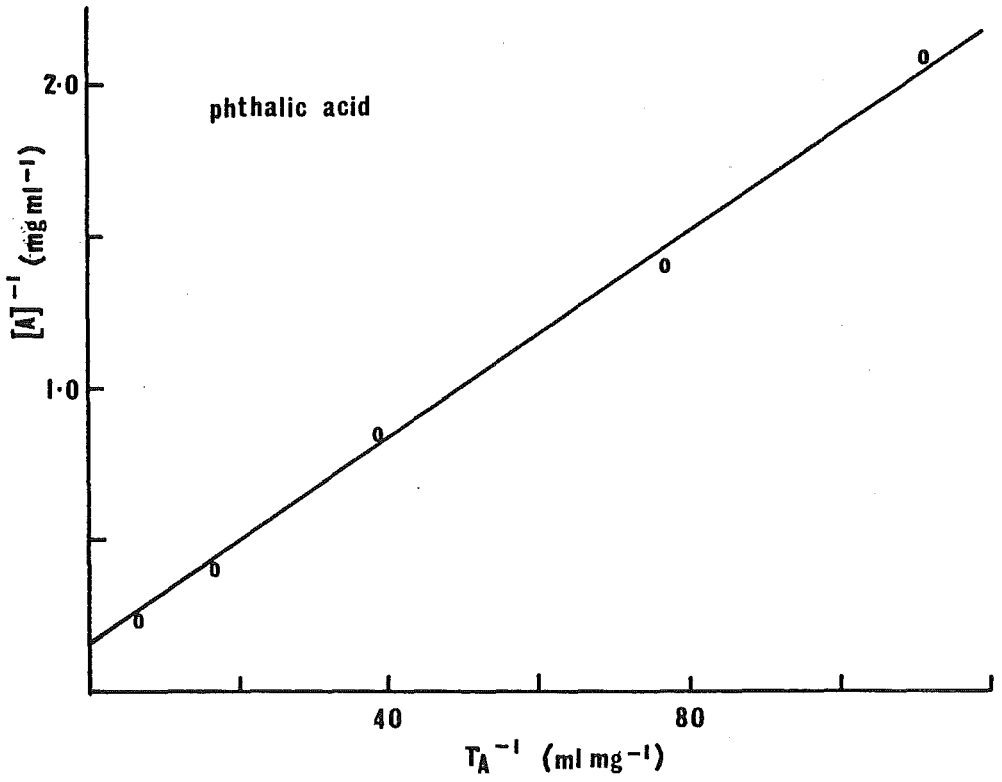
Langmuir Isotherms

Phthalic acid adsorption at pH 1.7 and desorption at pH 6.5 on XAD-7 followed Langmuir isotherms.

A Langmuir adsorption isotherm describes the equilibrium relationship between the concentration of adsorbed and solution species

$$\frac{1}{T_A} = \frac{1}{K[A]T} + \frac{1}{T}$$

where T_A is the equilibrium concentration of analyte on the resin (mg $m\ell^{-1}$), [A] is the concentration of analyte in solution, K is the equilibrium adsorption constant, and T is the maximum adsorptive capacity.



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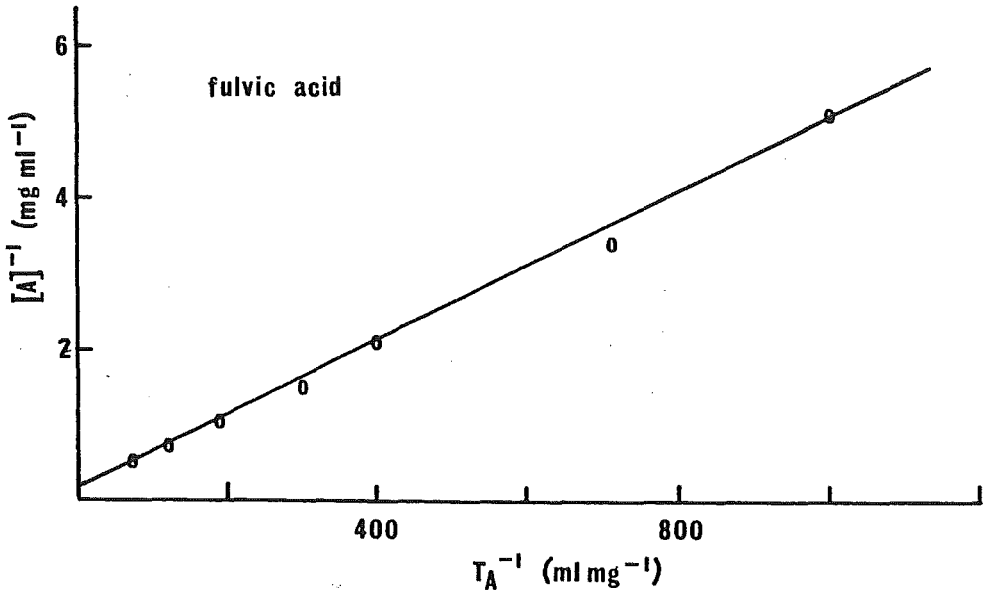


Figure 6.4a Inverse Langmuir adsorption isotherms for phthalic acid and fulvic acid (FA1) on XAD-7 resin, at pH 1.7.

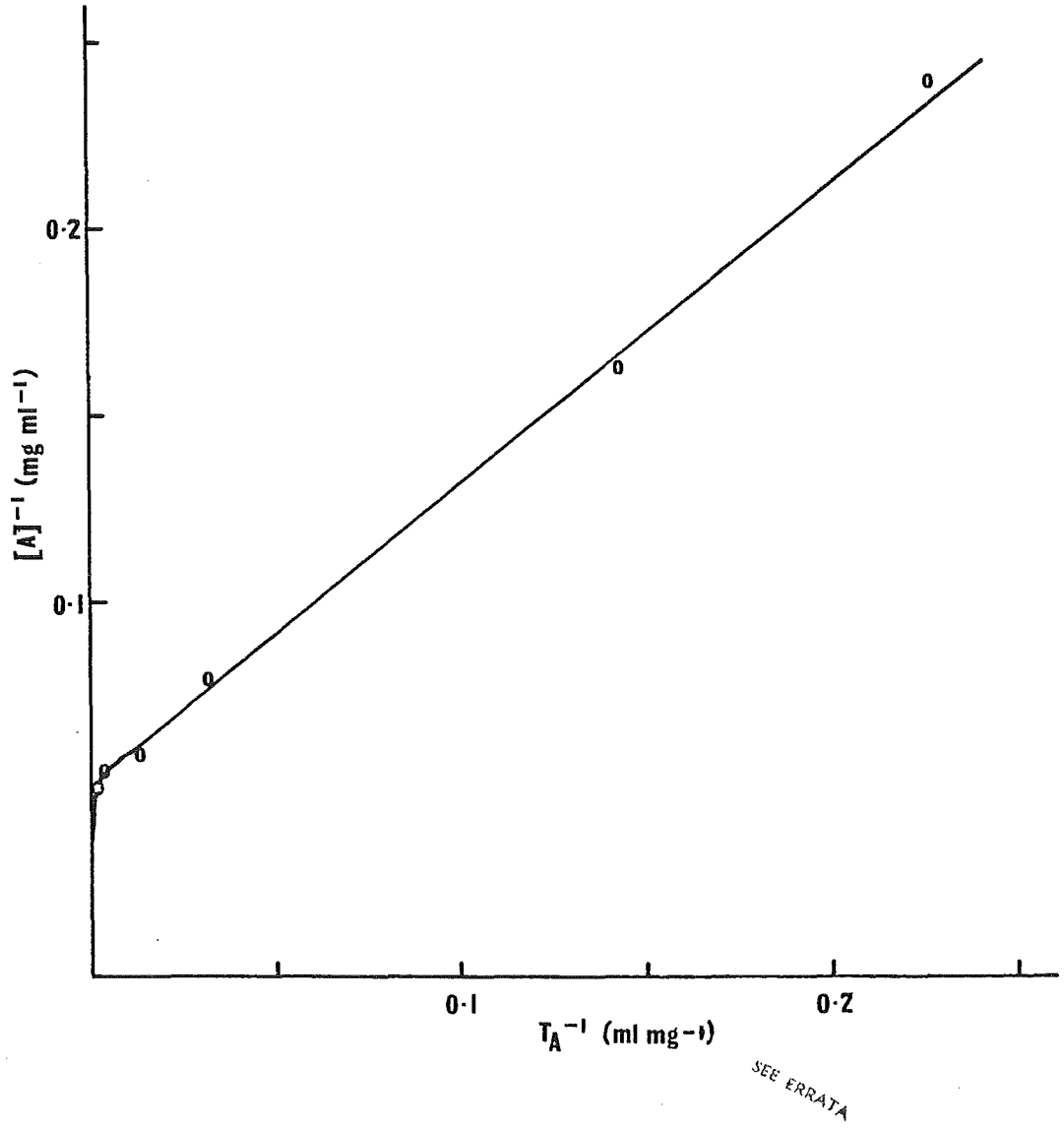


Figure 6.4b Inverse Langmuir desorption isotherm for fulvic acid (FA1) from XAD-7 resin, at pH 6.5.
Resin loading at pH 1.7 : 18.7 mg fulvic acid/10 mℓ XAD-7 resin.

A plot of T_A^{-1} vs. $[A]^{-1}$ will give a straight line with intercept T^{-1}, and K can be calculated from the slope.

For phthalic acid adsorption, T and K were calculated as 6.7 mg $m\ell^{-1}$ and 8.3 $m\ell$ mg^{-1} respectively (Figure 6.4a).

6.5.3 *Behaviour of Fulvic Acid on XAD-7*

Aiken et al. (1979) reported that equilibrium saturation of XAD-7 required 8 h for a fulvic acid sample at pH 2. In this work it was observed that freshly filtered (0.025 μ) solutions reached equilibrium within 30 min; it is deduced that Aiken's sample contained colloidal material. Further, when fulvic acid was adsorbed from coarsely filtered (0.45 μ) solutions, release was incomplete. At pH 6.5 a coarsely filtered solution released 74% and a finely filtered solution released 93%. It appears that the XAD resin acted as a filter, trapping colloidal material. The resin remained discoloured and was not readily regenerated.

Adsorption of fulvic acid on XAD-7 resin followed a Langmuir isotherm (Figure 6.4a). T was calculated as 35 ± 10 mg $m\ell^{-1}$, and the value of K was 5.6 $m\ell$ mg^{-1}.

In contrast, desorption of fulvic acid at pH 6.5 did not follow a Langmuir isotherm (Figure 6.4b). Regardless of the initial resin loading (0.5 - 2.0 mg $m\ell^{-1}$) and regardless of the resin pretreatment (solvent extraction with acetone then methanol, or acetone, methanol and dichloromethane) a proportion of fulvic acid (0.04 - 0.05 mg fulvic acid $m\ell^{-1}$) could not be removed from the resin at infinite dilution in solution at pH 6.5 or 13. This retention was not observed for phenols, or salicylic, citric or phthalic acids, and it was deduced that it arises from the existence of a component

in the fulvic acid with specific affinity for strongly adsorbing sites in the resin.

The residual organic matter ($\underline{c}$ 0.05 mg $m\ell^{-1}$), in the context of a soil extraction, is considered negligible. In soil extractions where average resin loadings of $\underline{c}$ 10-20 mg $m\ell^{-1}$ were involved, > 98% desorption was achieved at pH 6.5 with an additional 1% desorbed at pH 11.0.

6.6 ACID STABILITY OF FULVIC ACID SOLUTIONS

The amount of fulvic acid recovered from XAD-7 at pH 6.5 was found to be dependent on the contact time with the resin at pH 1.7 (adsorbed from a 0.025 μ filtered solution). For example, for a contact time of 2 h at pH 1.7, 97% of a fulvic acid sample was released at pH 6.5 after four batch extractions. After 40 days contact at pH 1.7, only 83% was released. This change cannot relate to precipitation of humic acids within the resin as these acids are sufficiently soluble at pH 6.5 to be extracted. However, the result does suggest that either a kinetic effect (the fulvic acid molecules penetrating further into the resin with time), or fulvic acid reacting with the resin, or fulvic acid being chemically modified (possibly by polymerization) at low pH, is responsible for the low recovery after prolonged contact with XAD-7 resin.

The stability of acidic fulvic acid solutions was studied by Sephadex gel chromatography. Solutions of fulvic acid aged in 0.1 M pyrophosphate at pH 1.7 and pH 3.5 were filtered (0.025 μ), adjusted to pH 9, applied to a Sephadex G-50 column (1 cm x 34 cm) and eluted with 0.01 M borax solution. Chromatograms for pH 1.7 solutions aged 7 and 30 days are shown in Figure 6.5. The older sample shows a

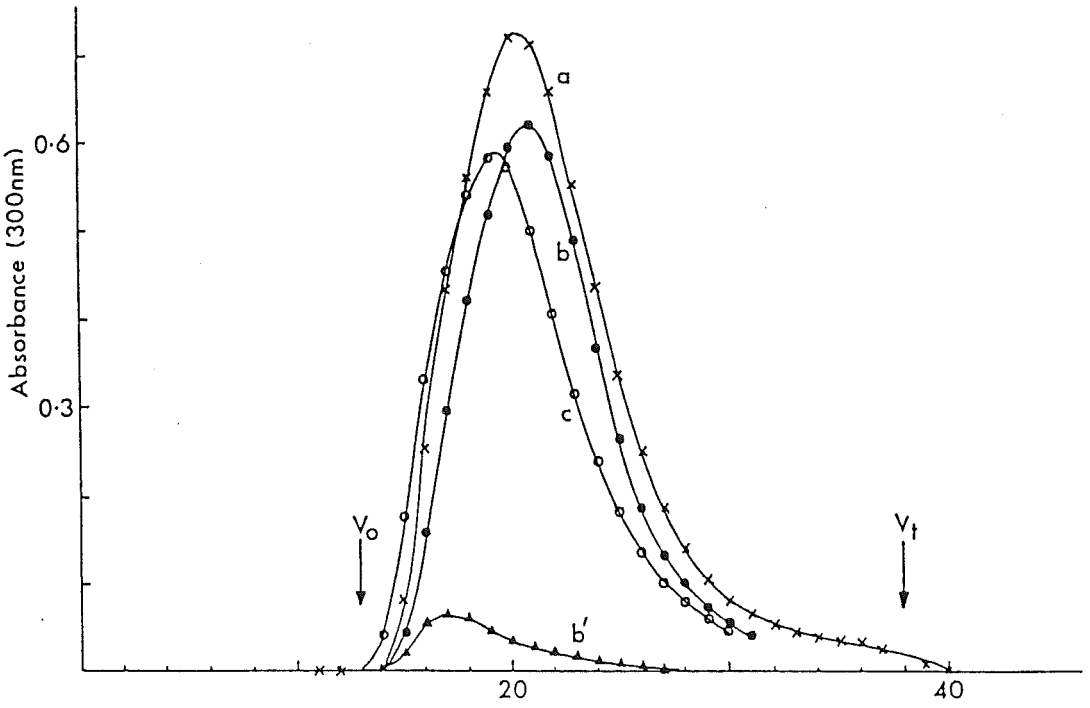


Figure 6.5 Sephadex G50 chromatogram for peat-derived fulvic acid aged in 0.1 M pyrophosphate at pH 1.7. a, t=0 ; b, t=7d (b' is precipitate removed at 7d) ; c, t=30d. Column 10x 340 mm, eluant 0.01 M borax, pH 9.18.

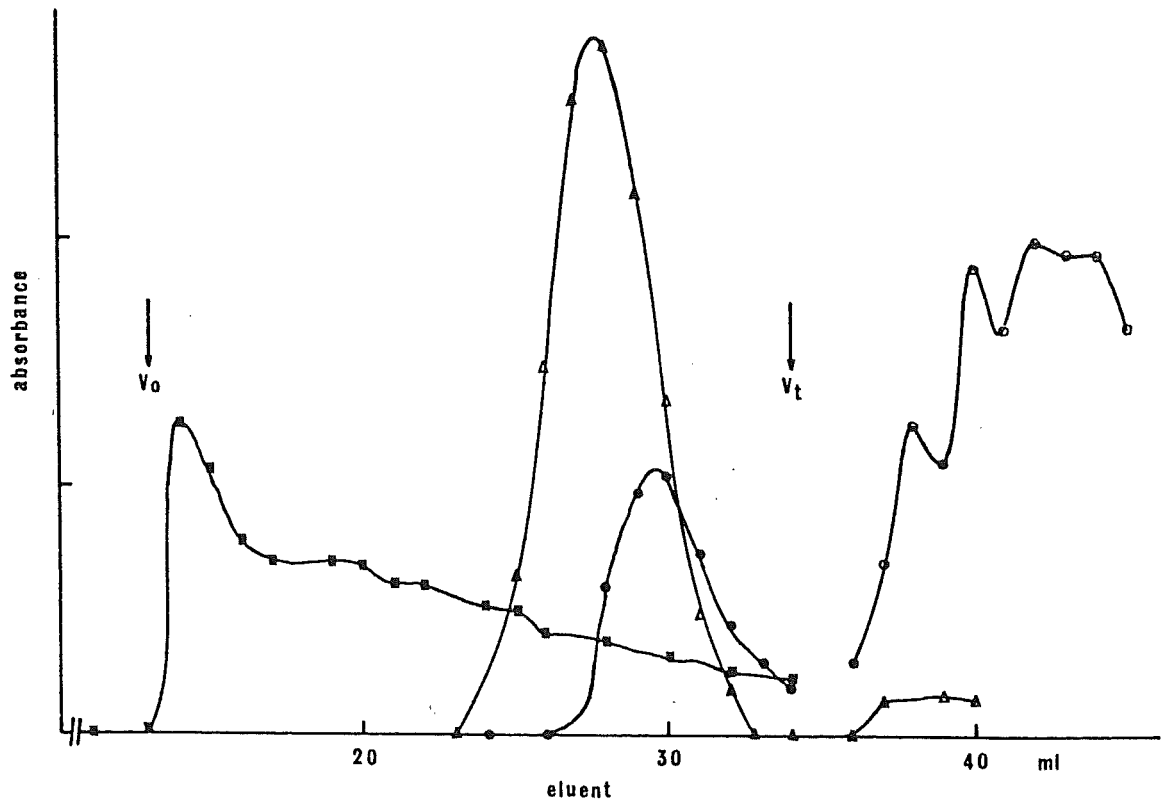


Figure 6.6 Attempted molelcular weight calibration of Sephadex G50 column. ■, Epicatechin polymer (mass average molecular weight c 4600) ; Δ, chromazurol S (molecular weight 539.3) ;

general increase in molecular weight, which is consistent with possible polymerization reactions. No change in molecular weight distributions was observed up to 14 days. However, after 5 days a brown precipitate had developed and this represented a higher molecular weight fraction than in the solution. No change in molecular weight was observed for fulvic acid in solution at pH 3.5 for up to 14 days. Thus, to maintain the integrity of fulvic acid samples it is essential that they are not stored in solution at low pH. During the extraction process, fulvic acid solutions were stored no longer than 18 h at pH 1.7 and for only three days at pH 3-4.

A molecular weight calibration of the Sephadex G-50 column was attempted using organic molecules with similar functional groups to those postulated for fulvic acid, and of known molecular weight (chromazurol S, Mω 539.3; tannic acid, Mω 1701; cytochrome C, Mω 12600). However, the molecules differed sufficiently in their functional group compositions and therefore specific affinity for the gel to elute at similar volumes despite their widely differing molecular weights (Figure 6.6).

6.7 PURIFICATION

6.7.1 *Centricon Filtration*

Centricon centrifugal microconcentrators were tested for removal of fine colloidal material from fulvic acid solutions prior to freeze drying. These concentrators contain low-adsorbing, hydrophilic membranes, with either 10000 or 30000 molecular weight cut-off. The intention was to make use of the fine membranes as ultrafilters, retaining

colloidal material above the membrane. However, when a sample of fulvic acid was centrifuged at 3000 rpm for 3 h in a Centricon-30 microconcentrator (30000 molecular weight cut-off), only 66% of the organic matter passed through the membrane (as determined by visible absorbance at 465 nm). Repetitive dilution and centrifugation of the retentate allowed a further 13% of the organic matter through the membrane, but 21% was retained above the membrane. Molecular weight distributions of the filtrate and retentate on Sephadex G-50 indicated that the filtrate was lacking in high molecular weight components, while the retentate contained a greater proportion of the higher molecular weight organic matter. This method for removal of colloidal material from fulvic acid solutions was considered unsatisfactory as it fractionated the organic matter on the basis of molecular weight or molecular size.

6.7.2 *Repeated XAD-7 Treatment*

The final products of acid pyrophosphate extraction had ash contents in the range 0 - 1.3% but typically < 0.6% (see Table 7.1).

Fulvic acids isolated by the traditional alkali/ion exchange method are variously reported to contain 2 - 10% ash, even following repeated dialysis or HF-HCℓ treatment. The high ash content arises not only from appreciable amounts of mineral matter dissolved during extraction, but also from the salts formed by neutralization of the base used for extraction.

Samples isolated on XAD resins commonly have only 1 - 2% ash (Thurman, 1985) because ions in an extractant solution or ions co-extracted from the soil are rejected by

the resins.

The low ash contents (< 0.6%) obtained in this work were achieved after only one XAD resin treatment. After a second XAD-7 treatment (re-adsorbing fulvic acid on XAD-7 from 0.1 M $H_2P_2O_7^{2-}$, steps (v) through to (ix) of Scheme 6.1), the total ash content of a gley-podzol fulvic acid was reduced from 0.6% to 0.2%. Significant decreases in the phosphate content (0.11% reduced to < 0.02%) and iron content (0.07% reduced to 0.01%) contributed to this.

CHAPTER 7

GENERAL PROPERTIES OF FULVIC ACID

7.1 INTRODUCTION

Fulvic acids contain a heterogeneous array of organic molecules, composed substantially of carbon, oxygen, hydrogen and nitrogen. By definition, they contain no

7.2 INORGANIC ASH CONTENT

During the extraction of fulvic acids, considerable quantities of inorganic constituents are also receovered (salts, sesquioxides, clay). These impurities must be removed before the organic matter can be properly characterized. During the removal of such impurities, the organic matter may be altered or lost. An ideal extraction method would result in a product that was free of inorganic constituents and did not require further purification. SEE ERRATA

It is not uncommon for fulvic acid extracts to contain 2 to 10% ash. High ash contents not only lead to ambiguities in elemental analyses (the result of error in total weight of organic matter), but also cause problems when studying acid - base properties and metal complexing. High ash contents may lead to severe drifting of pH in fulvic acid-base titrations and erroneous total metal concentrations in metal - ligand systems.

The total ash contents for the four fulvic acid samples extracted by the acid pyrophosphate/XAD-7 method (FA1, FA2, FA3, FA4) are reported. The composition of the inorganic ash has been determined by inductively coupled plasma spectroscopy (ICP) and atomic absorption spectroscopy (flame and carbon furnace AA).

7.2.1 *Total Ash Content*

The total ash contents for FA1, FA2, FA3, and FA4 fulvic acid samples are presented in Table 7.1. The ash contents were determined by igniting fulvic acid samples at 450^{o}C for three hours in a muffle furnace.

Table 7.1 Inorganic Ash Content and Elemental Analyses (%) of Fulvic Acids

	Total Ash	Al	Ca	Fe	K	Na	P	Si
FA1	0.0 (±0.2)	0.04	0.02	0.01	-	0.04	0.08	0.00
FA2	0.6	0.02	0.02	0.07	-	0.03	0.11	0.09
FA3	1.3	0.03	0.02	0.02	-	0.05	0.12	0.81
FA4	0.2	0.01	0.01	0.05	0.05	0.02	0.06	0.06

All fulvic acid samples extracted by the acid pyrophosphate/XAD-7 method contained considerably less than 1% ash, except for FA3. These fulvic acids contain much less ash than those samples extracted with alkali, i.e. FAI and FAS, or acid, i.e. FAA (see Chapter 3). The lower ash was achieved after only one treatment on XAD-7 resin. However, the ash content can be further lowered by a second XAD treatment (see Chapter 6).

The fulvic acid sample FA3 was extracted from a B_s soil horizon. This soil horizon is rich in amorphous inorganic components and these may account for the high ash content. The composition of the ash is discussed in Section 7.2.2.

7.2.2 *Ash Composition*

The composition of the ash in the fulvic acid samples is given in Table 7.1. Aluminium, calcium, iron, sodium, phosphorous and silicon analyses were performed by ICP spectroscopy. The potassium content, a possible contaminant from the extraction procedure, was determined by flame AA.

Carbon furnace AA was used to determine aluminium and iron concentrations; the results compared well with the ICP measurements.

For all acid pyrophosphate/XAD-7 extracted samples, the contribution to the ash from aluminium and iron was low. This is a necessary requirement for studies of metal-ligand and proton-ligand equilibria. High concentrations of these ions, which can bind with the fulvic acid, would result in misleading results. Some of the stronger binding sites would be occupied by aluminium and iron ions, thus limiting complexing by added metal ions.

The high silicon content of FA3 and the high iron and aluminium content of FAA resulted in severe pH drifting during acid-base titrations. As a consequence of this, no further studies were made on these samples.

The contribution from inorganic phosphorous (c 70% of total P) to the ash content of all acid pyrophosphate/XAD-7 fulvic acids was low ($\leq$ 0.1%). This highlights the efficiency of the XAD-7 process. During this extraction, c 2 ℓ of 0.1 M $Na_2H_2P_2O_7$ was passed through a soil column, then concentrated to c 100 mℓ prior to XAD-7 treatment. During the concentration step some pyrophosphate was precipitated. However, a high concentration remained in solution; this was efficiently separated from the organic matter during the XAD-7 adsorption/desorption process.

7.3 ELEMENTAL AND FUNCTIONAL GROUP COMPOSITION

The elemental analyses for FA1, FA2, FA3 and FA4 are given in Table 7.2. The compositions compared well with those reported in the literature (see Section 1.2.1).

Table 7.2 Elemental Analysis (%) of Soil Fulvic Acid

	C	H	N	O[a]
FA1	53.5	3.8	-	-
FA2	50.1	3.8	0.6	45.5
FA3	48.8	4.0	0.6	46.6
FA4	49.3	3.7	2.3	44.7

a by difference, 100% - (%C + %H + %N)

An obvious difference between the samples is the high nitrogen content of FA4. This value compares with the high nitrogen content of FAI. The high nitrogen content of these two samples is one of the factors used to explain the major differences in chemistry (proton and metal binding) between these samples and the other fulvic acids.

Approximately 50% of the nitrogen in fulvic acids has been accounted for by recognisable nitrogen-containing functional groups (Schnitzer, 1985). The climatic conditions under which the humic substances form has a profound influence on the distribution of nitrogen within these classes. However, the dominant proportion (30-40%) is ascribed to amino acids. Amino sugars and ammonia make up the remainder of the accountable nitrogen. At least 50% of the nitrogen remains unaccounted for.

The major oxygen containing groups in fulvic acids are carboxyl, alcoholic, carbonyl and phenolic groups. The

total carboxyl acidity was determined by titration of aqueous samples with standardized alkali. The equivalent weights (weight/mole of titratable carboxyl group) for FA1, FA2, FA4, FAI, FAH, and FAS were 160, 143, 139, 137, 131 and 124 (± 3%) respectively.

The lower the equivalent weight, the greater the density of carboxyl groups. Fulvic acids have lower equivalent weights than humic acids. Typically, fulvic acids have equivalent weights in the range 100 - 200 (Stevenson, 1982). It is difficult to compare values for samples from different origins. However, it is possible to compare the values for FA4, FAI and FAH because they are all extracted from the same soil. These three values are compared and discussed further in Section 7.5.3.

7.4 SPECTROSCOPIC CHARACTERISTICS

7.4.1 *Ultraviolet and Visible Absorption Spectra*

Fulvic acid solutions appear yellow to dark brown, depending on the concentration of the solution. Visible absorption spectra (400 - 800 nm) of fulvic acids are generally featureless, increasing in absorbance with decreasing wavelength. The absorbance at any given wavelength is dependent on the pH of the solution. As the pH is increased, the intensity increases.

Absorption spectra in the ultraviolet region (200 - 400 nm) are equally featureless. Absorption in this UV region arises from electron transitions involving multiple bonded units and lone pair electrons, e.g. C=C, C=O.

7.4.2 *Infrared Absorption Spectra*

The infrared absorption spectra of fulvic acids were recorded for samples pressed into KBr discs. IR spectra for FA4 are shown in Figure 7.1. Two spectra are given: (I) FA4, and (II) FA4 after heating at 120°C for 7 h in a KBr disc.

The major absorption bands in Figure 7.1(I) are labelled (a) to (h), and are assigned in Table 7.3.

Table 7.3 Assignment of IR Bands for Fulvic Acid

Band	Frequency (cm^{-1})	Assignment
(a)	3600 - 3200	O-H stretch of free water, alcohol, carboxylic acid. N-H stretch of amine.
(b)	2950	aliphatic C-H stretch.
(c)	2600	O-H stretch of carboxylic acids; NH_3^+ stretch of amino acid.
(d)	1710	C=O stretch.
(e)	1620	C=C; water O-H; amino acid NH_3^+ deformation.
(f)	1400	C-H deformation (aliphatic); O-H deformation; C-O stretch (carboxylic acid).
(g)	1220	C-H deformation (aromatic); C-O stretch and O-H deformation (carboxylic acid).
(h)	1050	polysaccharide C-O stretch; inorganic impurities.
(i)	2350	CO_2.

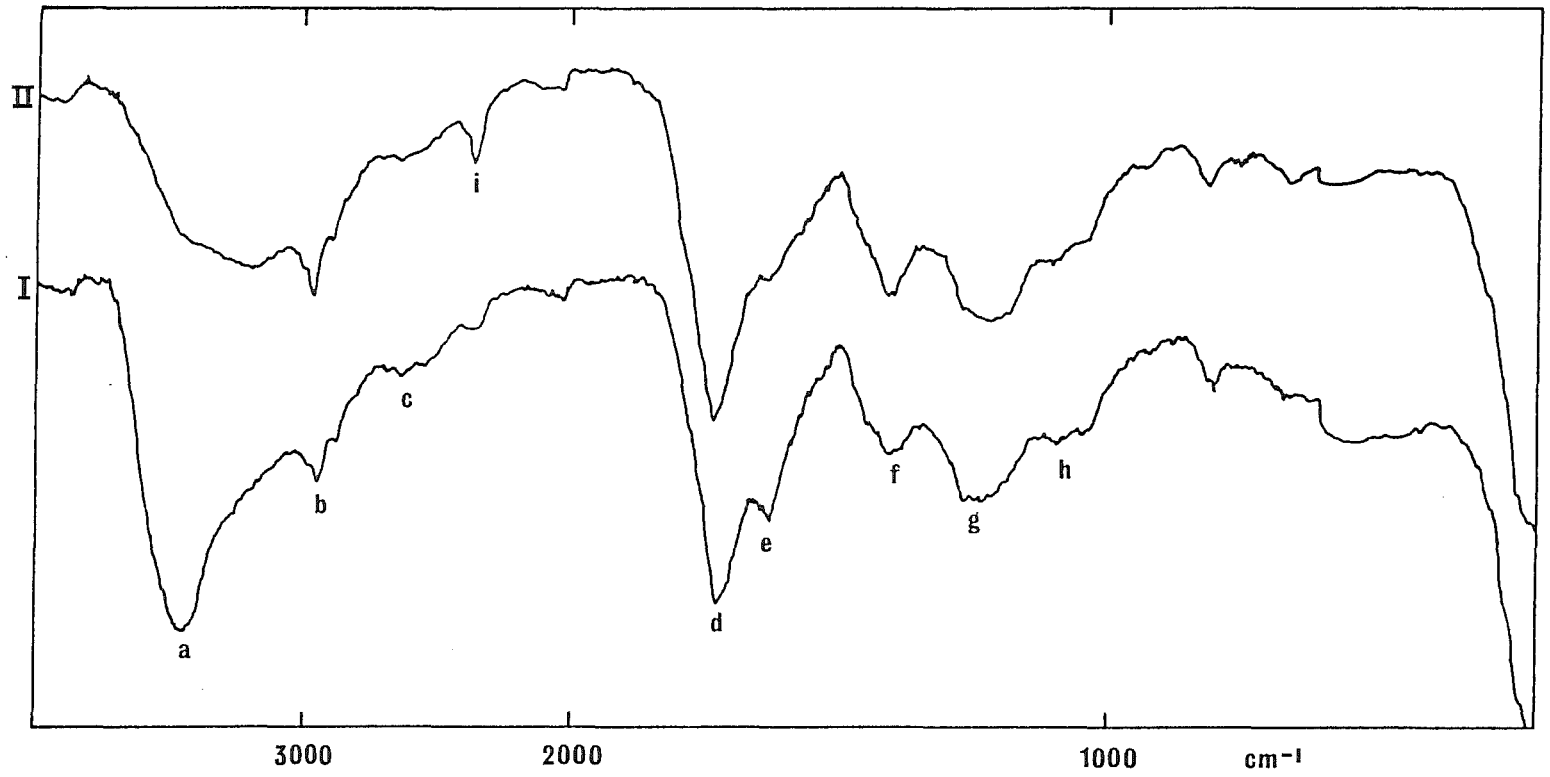


Figure 7.1 Infrared absorption spectra of fulvic acid (1 mg FA4 in 500 mg KBr). Spectrum (I) before heating. Spectrum (II) after heating at 120°C for 7 h in KBr disc. Refer to Table 7.3 for assignment of peaks.

The IR spectra reflect the preponderance of oxygen-containing functional groups, such as carboxyl, carbonyl and alcoholic groups. The strong absorption band at 3600-3200 cm^{-1}, and the strong band near 1710 cm^{-1} reflects the high carboxylic acid content of these humic substances.

The IR spectra for the other fulvic acids studied were very similar to Figure 7.1(I), except for the 1050 cm^{-1} absorption band. This band was stronger and more resolved for FAI. The band may be assigned to inorganic impurities, and would correlate with the high ash content of the sample. However, this band may also be assigned to polysaccharide (Stevenson, 1982).

An often encountered problem in IR absorption spectroscopy (especially when using KBr discs) is the interference caused by moisture. Water produces bands in the 3000-3600 cm^{-1} region and the 1600-1700 cm^{-1} region. The water may arise from the KBr or from the fulvic acid. Both should be dried prior to pressing discs. Fulvic acid may be dried under vacuum at elevated temperature; refluxing ethanol (78° C) was used to thermostat the drying cell in this work. KBr can be dried in an oven at temperatures greather than 100° C for several hours. Theng and Posner (1967) dried samples under vacuum at 75° C for 30 minutes in the die before pressing the disc. Stevenson and Goh (1974) heated KBr/fulvic acid discs for 2 h at 100° C, but recommended that prolonged heat should be avoided.

SEE ERRATA

Prolonged heating at temperatures greater than 100° C may lead to alteration of the organic matter. At the extreme, the organic matter may decompose to produce CO_2 and H_2O. Under less severe conditions, reactions such as anhydride

formation (from carboxylic acids) and decarboxylation of β-keto acids or β-dicarboxylic acids may alter the fulvic acid.

Wright and Schnitzer (1961) observed new bands at 1840 and 1775 cm^{-1} when fulvic acid was heated (170° C) for 50 h. These bands were ascribed to probable formation of cyclic anhydrides.

Figure 7.1(II) is the IR spectrum for FA4 heated at 120° C for 7 h. The spectrum after 22 h was essentially the same, i.e. there was no evidence for further decomposition.

The most obvious change following heating is the decrease in the intensity of band (a). This is probably due to the loss of water from the KBr disc. Associated with this change is the decrease in intensity of band (e), also associated with water. The remaining intensity in the region 3600-3200 cm^{-1} is assigned to O-H stretching of carboxylic acids and alcohols, and to amine N-H stretching.

Another noticeable change in the IR spectrum following heating is the appearance of a sharp peak at 2350 cm^{-1} (peak (i), Figure 7.1(II)). This peak can be assigned to the presence of CO_2. The presence of CO_2, trapped in the KBr disc, may arise from decarboxylation reactions of β-keto acids or β-diacids (Streiwieser and Heathcock, 1976). Further support for a decarboxylation reaction may be the relative increase in intensity of bands (b) and (f), both being assigned to aliphatic C-H.

These decarboxylation reactions were demonstrated for model compounds. For malonic acid and alkyl substituted malonic acids, decarboxylation reactions are apparent after several hours heating at 100-180°C, i.e.

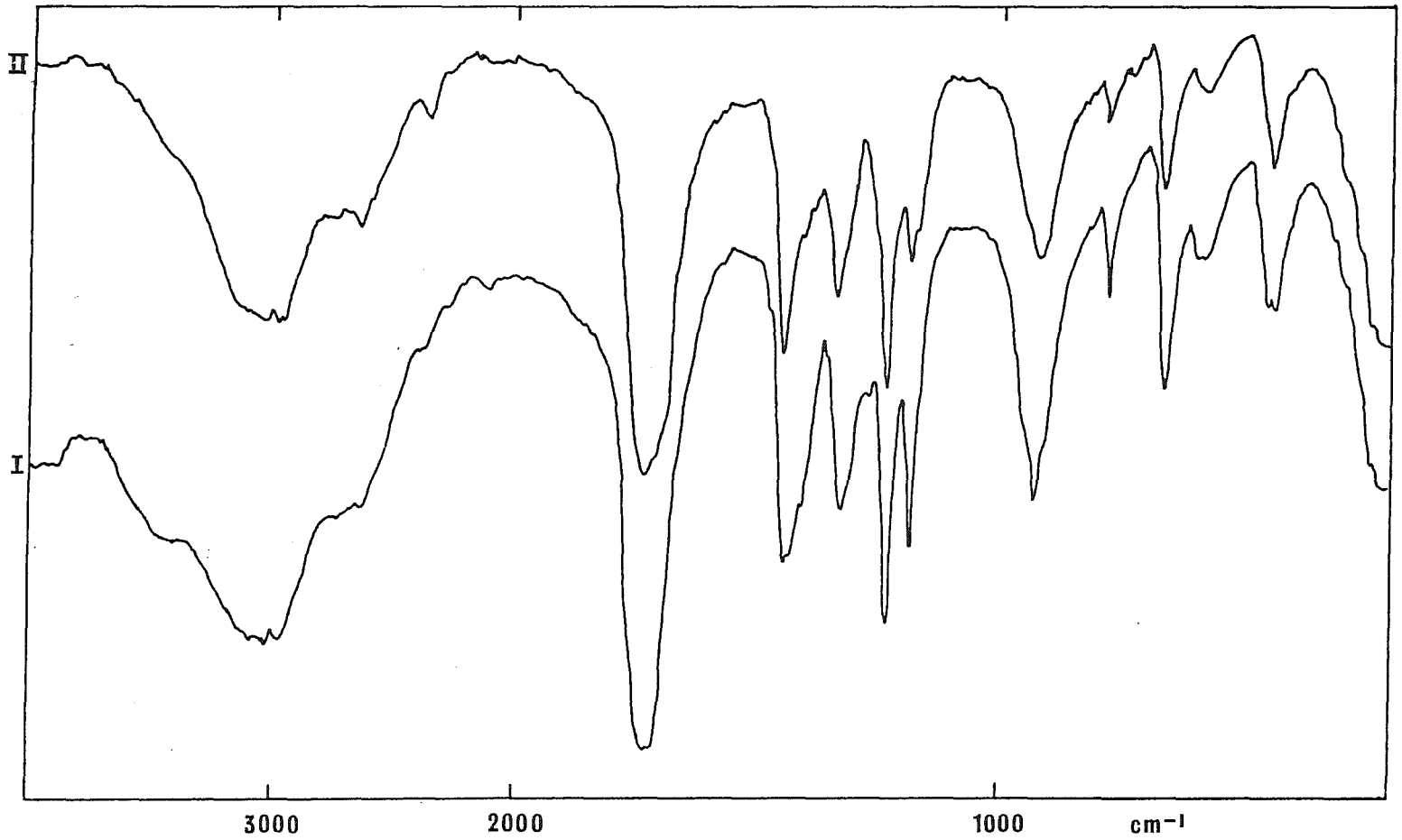
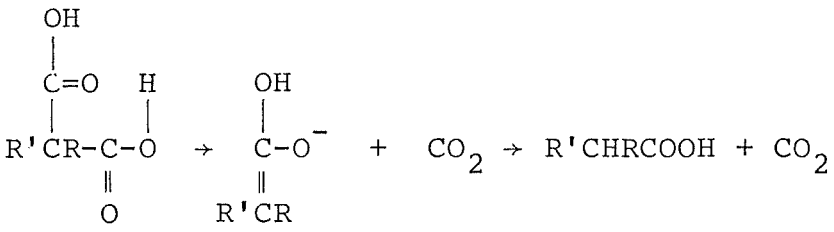


Figure 7.2 Infrared absorption spectra of malonic acid. Spectrum (I) KBr disc dried at 80°C for 1 h. Spectrum (II) after heating at 100°C for 2.5 h in KBr disc.

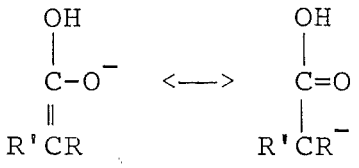


The net result is the loss of carboxyl group and replacement by a proton, and the evolution of CO_2. The effects of these changes can be seen in the IR spectra in Figure 7.2. Figure 7.2(I) shows the IR spectrum of malonic acid; the KBr disc was dried for one hour at 80° C. Figure 7.2(II) (heated for 2.5 h at 100° C) shows the sharp peak characteristic of CO_2 (2350 cm^{-1}). Changes have also occurred in the 1200-1400 cm^{-1} region, a region assigned to aliphatic C-H and O-H deformations. Decomposition beyond decarboxylation (i.e. to CO_2 and H_2O) is not indicated. However, after heating at 120° C for three hours, signs of decomposition were apparent.

For fulvic acid, decomposition beyond decarboxylation (CO_2 and H_2O) was not indicated because the IR spectra did not change beyond four hours heating, and no increase in the regions of H_2O absorption were observed (3000-36000 cm^{-1} and 1600-1700 cm^{-1}). SEE ERRAT.

Decarboxylation reactions at 100-180° C are generally limited to β-keto acids or β-dicarboxylic acids. These molecules readily decarboxylate because :

(i) When the carboxylate ion decarboxylates, it forms a resonance stabilized ion, i.e.



This is much more stable that the anion RCH_2^- that would form by decarboxylation of a monocarboxylic acid, or a polycarboxylic acid where the carboxyl groups are not β to each other.

(ii) When the acid decarboxylates, it can do so through a six-membered cyclic transition state, producing an enol directly and avoiding the anionic intermediate (Streitwieser and Heathcock, 1976).

γ-diacids (e.g. citric acid, tricarballylic acid) do not readily decarboxylate. A tricarballyic acid/KBr disc was heated at 120° C for five hours, and there was no evidence for decarboxyation.

δ-diacids (e.g. succinic acid) do not decarboxylate easily, but do form anhydrides. Anhydrides are characterized by peaks at 1760-1790 cm^{-1} and 1800-1850 cm^{-1}. There was no evidence for the formation of anhydrides in fulvic acid upon heating. No band was observed in the region 1800-1850 cm^{-1}. It was not possible to detect the anhydride peak in the region 1760-1790 cm^{-1} because of the broad nature of the carbonyl band (Figure 7.1(d)).

The appearance of a CO_2 peak upon heating fulvic acid suggests that malonic acid units may be present in the organic structures.

7.4.3 *Nuclear Magnetic Resonance Spectroscopy*

While it is not possible to unambiguously interpret the nmr spectra for fulvic acids, it is possible to identify probable structural units.

The ^{1}H nmr spectrum was recorded on a Varian XL300 nmr instrument, for c 2 mg FA4 in D_2O. The proton resonance region at 0-5 ppm reveals that the fulvic acid is highly aliphatic. The peak at c 0.9 ppm is assigned to methyl protons (CH_3-). The peak between 1.2 and 1.8 ppm is assigned to methylene protons (-CH_2-), whereas the broader peak between 2.0 and 3.0 ppm is assigned to methyl and methylene protons in environments such as CH_3-COOR, CH_3-COOH, R-CH_2-NH_2, R-CH_2-COR. Methyl and methylene carbons substituted by alcoholic and ether groups give rise to the peaks in the region 3.5-4.0 ppm. The presence of olefinic protons may have been partially masked by the HOD peak. Olefinic protons give rise to resonaces between 4.7 and 6.0 ppm. The very broad, weak band centered around 7 ppm is the aromatic proton resonance band. It is inferred that the aromatic content is low, or that the aromatic groups are heavily substituted. The low aromatic content or presence of substantially substituted aromatic units is supported by the absence of a peak at 3100-3000 cm^{-1} (assigned to aromatic C-H stretch) in the IR spectrum (Figure 7.1).

The ^{13}C nmr spectrum (Figure 7.3b) showed four broad regions of resonance; 20-50 ppm (methyl and methylene carbons, amino acids), 70-90 ppm (carbons adjacent to oxygens, $-CH_2-O$, $\overset{|}{\underset{|}{C}}-O$, $-\overset{|}{\underset{|}{C}}-O$), 115-165 ppm (aromatic and olefinic carbons), 175-190 ppm (carboxyl carbons).

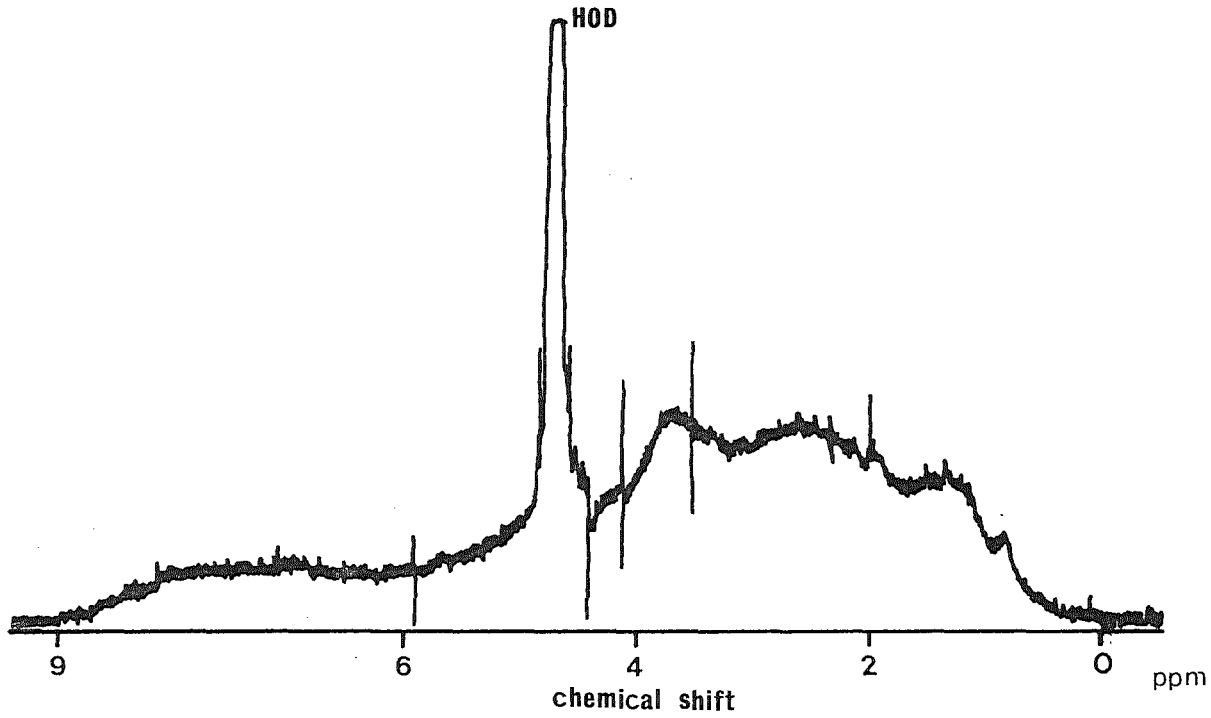


Figure 7.3a ^{1}H nmr spectrum of fulvic acid (FA4, 2 mg) in 0.5 mℓ D_2O, recorded on a Varian XL 300 nmr spectrometer.

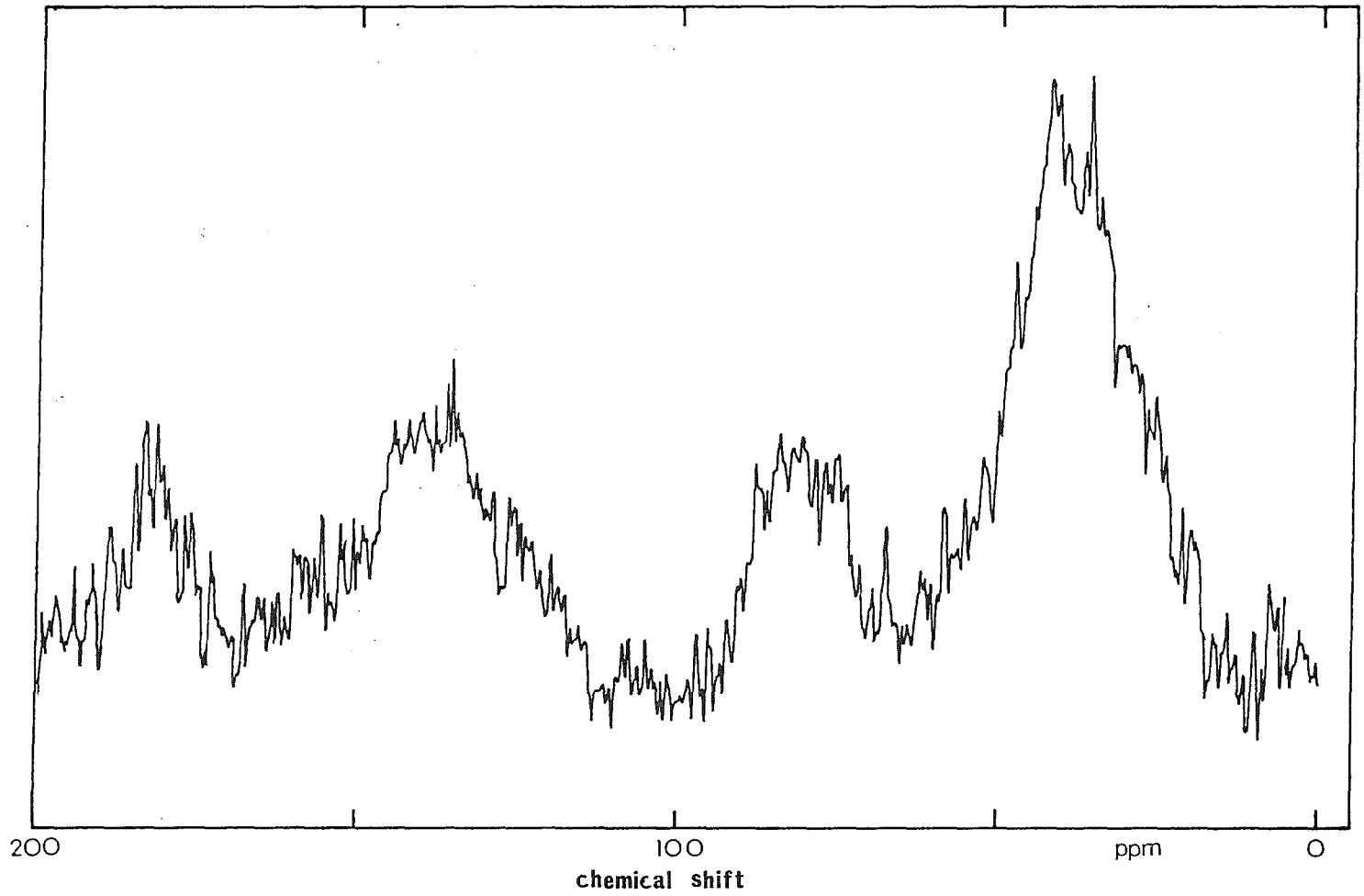


Figure 7.3b ^{13}C nmr spectrum of fulvic acid (FA1, 80 mg) in 0.4 mℓ D_2O, recorded on a Varian CFT-20 nmr spectrometer.

The aliphatic region of the ^{13}C nmr spectrum (0-100 ppm) indicates two distinct types of carbons, those giving resonances in the region 20-50 ppm and those in the region 70-90 ppm. Carbons in alkyl chains give rise to the intense, broad band between 20 and 50 ppm. Amino acid carbons may also contribute to the signal in this region (e.g. α-carbon of glycine, 42.6 ppm); reported resonances span the region 40-70 ppm.

There is little evidence for polysaccharides in the fulvic acid. These would produce signals in the 60 to 65 ppm region and the 90-105 ppm region. This result is consistent with the low polysaccharide content indicated by infrared studies (Figure 7.1, peak h).

In the 50-100 ppm region, aliphatic carbons adjacent to oxygen or nitrogen are usually observed. Amino acid carbons are expected to contribute to the signals between 40 and 70 ppm. The absence of signals in this region suggests that the amino acid content of this sample is low. A low amino acid content for this sample would be consistent with the low nitrogen content (0.6%).

It is difficult to infer the aromatic content of fulvic acid because the resonance for olefinic carbons overlaps in the same region of the spectrum (115-165 ppm).

The smaller peak at 175-190 ppm has been assigned to carboxyl carbons. Carbonyl carbons of aldehydes, ketones and esters may also contribute to this region.

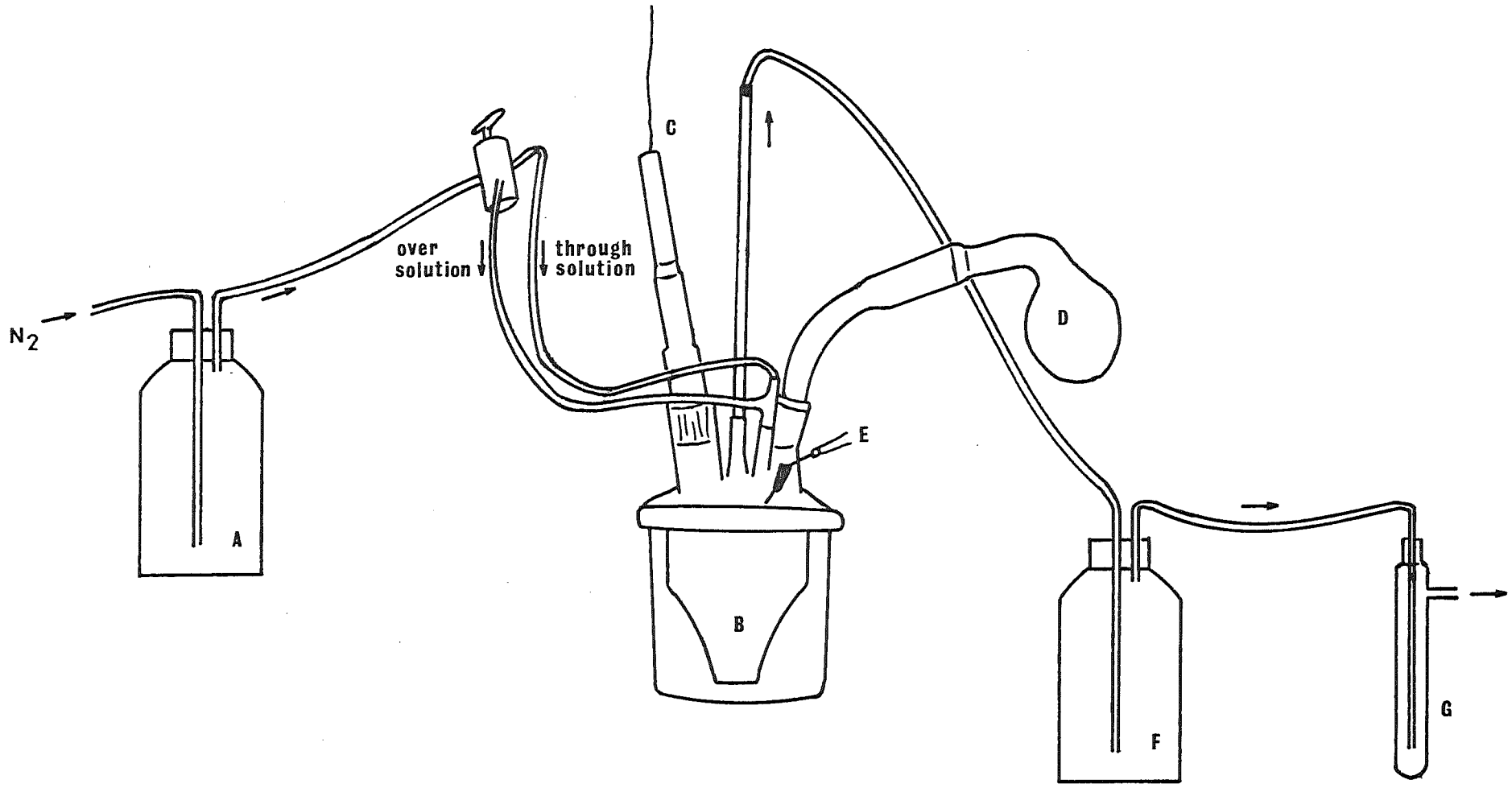


Figure 7.4 Closed system for fulvic acid hydrolysis. A, acidic vanadium(II) oxygen scrubber ; B, hydrolysis cell ; C, pH combination electrode ; D, cation exchange resin, H^+-form ; E, alkali syringe ; F, water trap ; G, deoxygenated KOH solution.

7.5 ALKALINE HYDROLYSIS OF FULVIC ACID

For studies on fulvic acids to be meaningful it is essential that the acids not be modified in the extraction process. This is most unlikely when alkali extraction methods are used. Phenolic compounds are known to oxidise in alkaline solution in the presence of oxygen (Mihailovic and Cekovic, 1971). Careful alkali extraction procedures will remove all oxygen prior to raising the pH. However, in the absence of oxygen, alteration of organic matter at high pH can still occur. For example, ester linkages may be cleaved, generating a fragment with a terminal alcoholic group and a fragment with a terminal acidic group.

The fulvic acid sample most appropriate for observing the effects of alkali treatment is FA4 (IHSS peat). This sample, and the resulting product of alkali treatment (FAH), can be directly compared with the IHSS alkali extracted fulvic acid (FAI); FA4 was extracted from the same peat sample as FAI, but using the acid pyrophosphate/XAD-7 method.

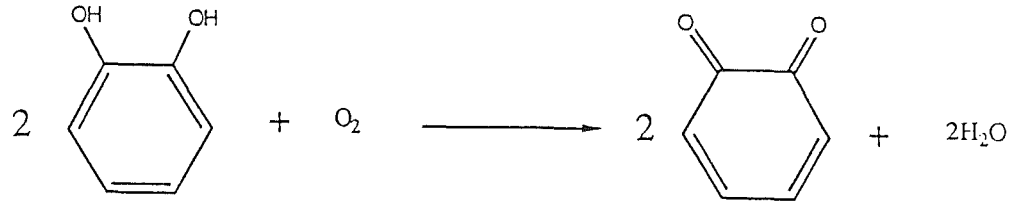
The molecular weights, equivalent weights and pK values for the three samples were determined. Hydrolysis products arising from possible ester hydrolysis were detected by g&c analysis.

7.5.1 *The Alkali Treatment*

Figure 7.4 is a schematic representation of the apparatus used to treat fulvic acids at high pH with the exclusion of oxygen.

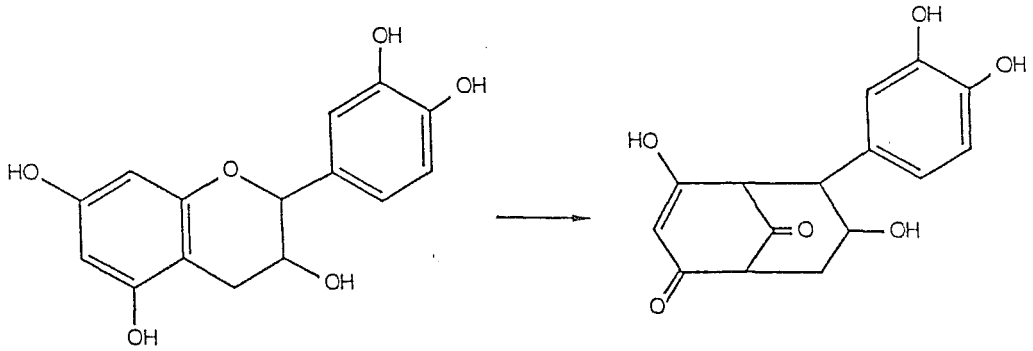
Complete exclusion of oxygen was established in separate studies using solutions of catechol (I) and epicatechin (II). These two compounds are extremely

sensitive to oxygen in alkaline solution. Catechol is oxidised to the quinone (III). The formation of the quinone is readily detected by changes in the UV spectrum. Catechol absorbs strongly at 274 nm ($\varepsilon \underline{c}$ 2400), whereas the quinone absorbs at 390 nm ($\varepsilon \underline{c}$ 1400) (Mason, 1949).



I III

Epicatechin is rapidly oxidised to catechinic acid (IV). Epicatechin absorbs at 278 nm ($\varepsilon \underline{c}$ 3920), whereas catechinic acid absorbs much more strongly at 285 nm ($\varepsilon \underline{c}$ 18600) (Kennedy et al., 1984).



II IV

The solution containing I or II was placed in the hydrolysis cell. The lid was sealed with silicon grease and clamped. An oxygen probe, a combination pH electrode, a nitrogen bubbler, and the vessel containing cation exchange resin (H^+ form) were sealed into the lid of the cell. The cell was flushed with oxygen-free nitrogen that had been passed through an acidic vanadium(II) oxygen scrubber, for two hours. The oxygen meter connected to the oxygen probe indicated that all oxygen had been removed. The pH was raised to 11 with deoxygenated KOH solution; KOH was introduced through a syringe sealed into the cell with a septum seal.

After 20 h at high pH, the pH was lowered to $\underline{c}$ 2.5 by addition of the cation exchange resin. At low pH the cell could be opened to the atmosphere without oxidation of the phenol. UV spectra were recorded for the solutions before and after exposure to high pH. No significant changes in the spectra were observed, indicating that the cell was completely sealed from the atmosphere and oxygen free while at high pH.

Two fulvic acids were exposed to high pH in the absence of oxygen (FA1 and FA4). The solutions (100 mℓ) contained $\underline{c}$ 100 mg of fulvic acid. For FA1, three separate experiments were performed where the solutions were maintained at high pH for 2, 24 and 72 h. For FA4, solutions were raised to high pH for 4, 24 and 72 h. Following exposure to high pH, the solutions were lowered to pH $\underline{c}$ 2.5 by addition of cation exchange resin. The recovered solutions were further cation exchanged (three columns of Dowex 50W-8X resin) prior to concentration (by rotary evaporation), filtration (0.025 μ)

and freeze drying. The product resulting from FA4 72 h exposure to high pH has been designated FAH.

7.5.2 *Molecular Weight Distribution*

The molecular weight distributions of FA4, FAH and FAI were compared using Sephadex G50 gel exclusion chromatography. An automated system eluted 0.01 M borax through a Sephadex G50 column (column volume 51.1 cm^3, void volume 20.4 cm^3) at 0.8 mℓ/min. Eluant was passed through a flow cell (2.5 mm) where the absorbance at 280 nm was continuously monitored. The chromatograms for 500 μℓ injections of fulvic acid solution (1 mg/mℓ in borax) are shown in Figure 7.5.

Sephadex G50 gel has a molecular weight exclusion limit of 10000 for dextrans and 30000 for peptides. Fulvic acid molecules with molecular weights greater than the exclusion limit are indicated (Figure 7.5); they elute at the void volume (V_o). The remaining molecules elute shortly after the void volume, indicating that they are also of high molecular weight or highly aggregated. Further, small molecules (less than the lower molecular weight cut-off for Sephadex G50) are not indicated; these would elute at a volume close to the bed volume (V_t). An interpretation in terms of highly aggregated fulvic acid molecules is consistent with the model for fulvic acid structure proposed by Wershaw (1986).

An uncertainty of ± 0.24 mℓ is associated with the elution volume at the absorbance maxima. The difference between elution volumes at the absorbance maxima for FA4 and FAH was 0.36 mℓ, and the difference between FA4 and FAI was

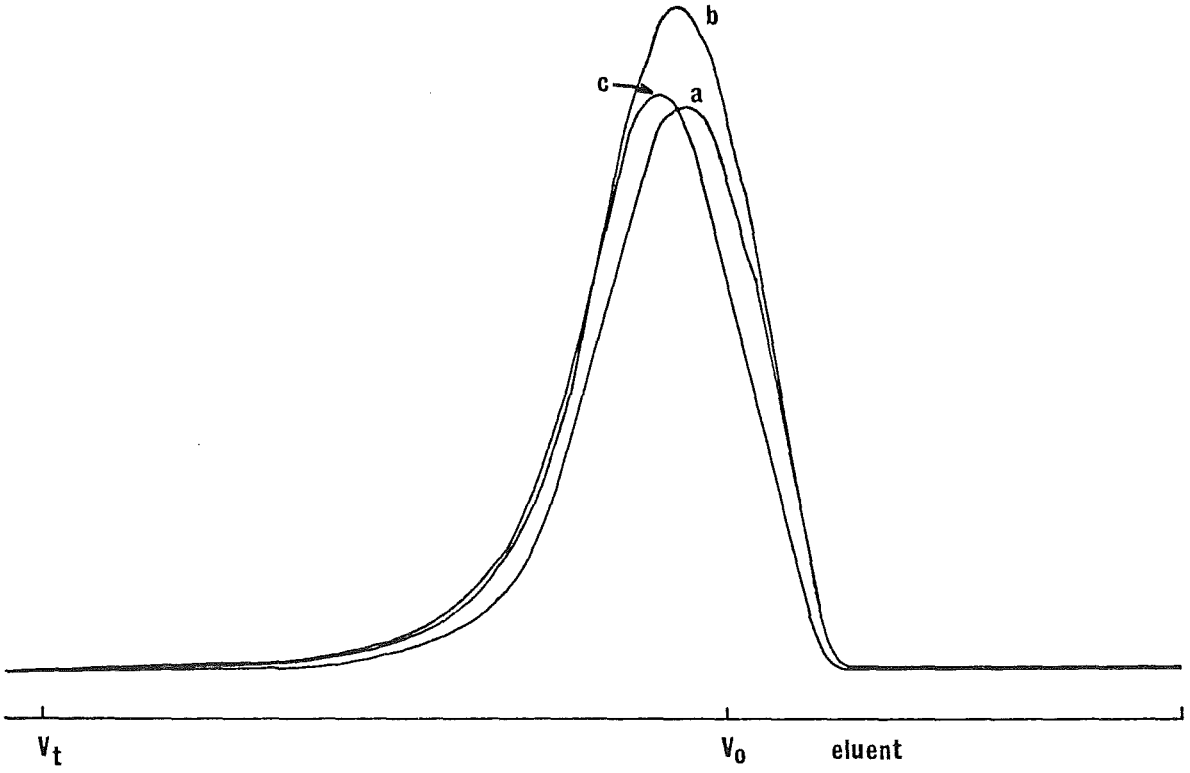


Figure 7.5 Sephadex G50 gel chromatogram for
(a) peat-derived fulvic acid,
(b) exposed to 0.1 M alkali for 72 h, and
(c) alkali extracted (IHSS) ;
eluent 0.01 M borax, pH 9.18.

0.84 mℓ. There was no significant shift in the elution volume between FA4 and FAH. However, FAI shows a significant shift in absorbance maximum to higher elution volume, i.e. to lower molecular weight.

The leading edge of the chromatogram (highest molecular weight region) for FAI was displaced to lower molecular weight when compared with FA4. This indicated a loss of the highest molecular weight molecules or highly aggregated molecules, and replacement with lower molecular weight or less aggregated molecules (as shown by the tailing edge of the chromatogram).

The absence of change in molecular weight distribution after alkali treatment (compare FA4 with FAH) need not indicate that fragmentation has not occurred. If an ester linkage occurs near the end of a large molecule, then loss of one fragment from the end of the molecule would not significantly change the molecular weight of the other fragment.

7.5.3 *Acid-base Properties*

(a) Equivalent Weight

The concentration of titratable carboxyl groups was determined by titration of the fulvic acid solution with standardized KOH. The solution was titrated beyond the end point (pH $\underline{c}$ 7.9), and the end point determined by a Grans plot (see Section 2.3).

The equivalent weights (weight per mole of COOH) of the alkali treated fulvic acids are compared in Table 7.4.

Table 7.4 Equivalent Weights of Alkali Treated Fulvic Acids

	Exposure Time (h)		
	0	4	72
FA1	163 ± 5		149 ± 4
FA4	139 ± 4	137 ± 4	131 ± 4

Alkali treatment (72 h) of fulvic acid caused a significant decrease (6-9%) in equivalent weight. This is consistent with an increase in carboxyl group density, as may arise from hydrolysis of ester linkages, i.e.

$$R\text{-}COOR' \rightarrow R\text{-}\underset{\underset{O}{\|}}{C}\text{-}OH + R'\text{-}O^{-}$$

A less prolonged reaction with alkali (4 h) did not produce a significant change in equivalent weight.

The IHSS peat fulvic acid (FAI) was exposed to alkaline conditions for $\underline{c}$ 16 h during its extraction. This fulvic acid sample had an equivalent weight of 137, which was not significantly different from the acid pyrophosphate/XAD-7 extracted sample (FA4).

(b) Protonation Constants

The fulvic acid titration curves were analysed in terms of a group of monoprotic acids with mean pK_n values (n=1-4). A detailed discussion of this numerical approach describing the acid-base titration curve is presented in Chapter 8.

Results in Table 7.5 show the mean pK_n values and the percentage (C_n) of carboxyl groups in each grouping of monoprotic acids for FA4, FAH and FAI.

Table 7.5 Protonation Constants of Alkali Treated Fulvic Acids

	log K_1	C_1(%)	log K_2	C_2	log K_3	C_3	log K_4	C_4
FA4	6.50	6	5.57	7	4.67	18	2.63	70
FAI	7.19	7	5.91	10	4.82	23	3.02	60
FAH	7.03	5	5.61	9	5.03	10	3.20	76

The effect of alkali treatment on molecular weight distribution was to lower the average molecular weight, consistent with an ester hydrolysis reaction. Consistent with this, the equivalent weight of fulvic acid was found to decrease upon exposure to alkaline conditions; a decrease in equivalent weight corresponds to an increase in carboxyl group density.

The predicted effect of an increased carboxyl group density upon the log K values was to decrease log K_n and increase log K_1. For example, log K_n for pentanedioic acid is 4.1, compared with 3.5 for 1,2,3-propane tricarboxylic acid (Perrin, 1979) which has a higher density of carboxylic functional group. Log K_1 is increased from 5.01 in pentanedioic acid to 5.9 in 1,2,3-propane tricarboxylic acid.

The observed effect of alkali treatment on fulvic acid

protonation constants (compare FA4 with FAH) was to increase log K_1 (+0.5), but also to increase log K_4 (+0.6).

One reaction which could contribute to this unexpected change in log K_4 is the formation of a peptide from condensation of an amino acid and an amino acid ester (Satchell and Satchell, 1969), i.e. :

$$RCH(NH_2)COOR' + R"CH(NH_2)COOH \rightarrow$$

$$RCH(NH_2)CONHCH(R")COOH + HOR'$$

Log K_n for amino acids such as glycine or alanine is 2.4. Peptide formation between these two amino acids results in a log K_n of 3.2, a similar change in log K to that observed for the fulvic acids FA4 and FAH. A less prolonged exposure to alkali in extraction of FAI caused an intermediate change in log K_4.

The elemental analyses of these fulvic acid samples indicate that they contain 2.3% nitrogen (Table 3.1 and 7.2). This is sufficient for one carboxyl group in five to be part of an amino acid.

The condensation reaction would produce a low molecular weight fraction (R'OH), consistent with Sephadex G50 chromatograms, and would also produce a higher molecular weight fraction.

7.5.4 *Glc Detection of Hydrolysis Products*

The most definitive way of establishing that a reaction has taken place is to isolate the products and characterize them. Thus, to establish that a hydrolysis reaction has occurred, the alcoholic and/or acidic fragments must be isolated and characterized. In this work, the alcoholic fragments were isolated on a C_{18} Sep-Pak cartridge and

analysed by capillary g&c.

A fulvic acid solution (FA4) was adjusted to pH 6.5 and non-ionic components removed on a Sep-Pak cartridge. The ionic eluant (i.e. the ionised carboxyl fraction) was hydrolysed at pH 13 for 72 h, as described in Section 7.5.1. The pH was lowered to 6.5 by addition of cation exchange resin to the closed system. The resulting non-ionic products from ester hydrolysis (i.e. alcoholic fragments) were recovered on the C_{18} Sep-Pak, and eluted with acetone. The acetone eluant was analysed by capillary g&c. (Figure 7.6).

A standard solution, containing 1 mg/m& each of phenol, vanillin and 1,5-dihydroxy napthalene, gave reference signals with retention times similar to those for fulvic acid hydrolysis fragments. The fulvic acid solutions contained phenol as an internal standard.

The presence of three non-ionic hydrolysis fragments is indicated. One (c) has an elution volume similar to vanillin, and another (a) similar to butanol or pentanol.

The products detected collectively represent only 0.3% of the sample mass. However, it is probable that the alcoholic fragment may also contain a number of ionic (carboxyl) groups which would render the fragment ionic at pH 6.5 and hence it would not be retained on the C_{18} Sep-Pak.

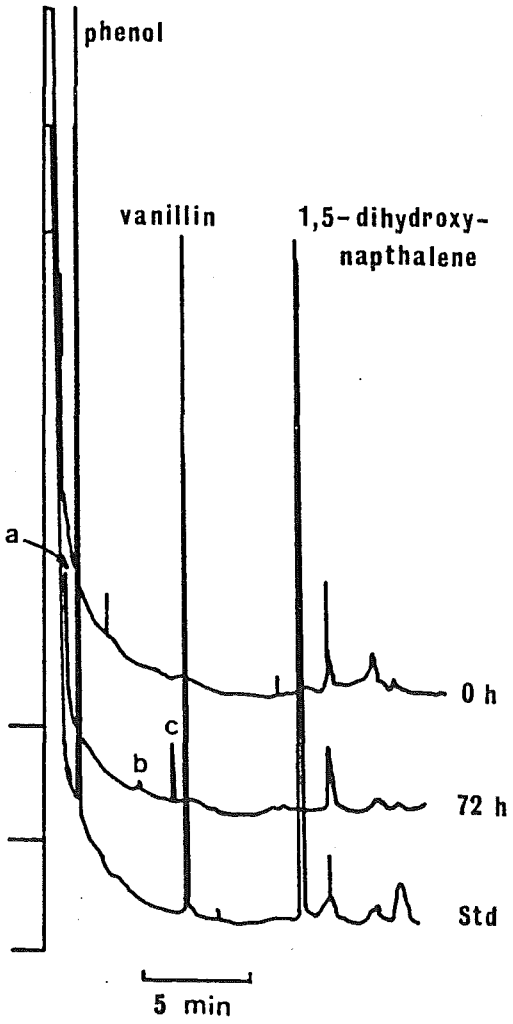


Figure 7.6 Gℓc traces of fulvic acid hydrolysis products in acetone.
Instrument = Shimadzu GC 9A with capillary column (30m x 0.75 mm ID).
Temperature program: 100-250°C at 10° min^{-1}, then 250°C.
Standards (Std) 1 mg $mℓ^{-1}$.

CHAPTER 8

ACID-BASE PROPERTIES OF FULVIC ACID

8.1 INTRODUCTION

The occurrence of many acidic functional groups in fulvic acids accounts for its solubility in aqueous systems and hence its mobility in the soil. The less soluble humic acids have a lower proportion of carboxylate groups and are sparingly soluble.

Another important function of the acidic functional groups is their ability to bind cations, either by electrostatic attraction (e.g. K^+, Na^+, Ca^{2+}, Mg^{2+}) or by complexing (e.g. Cu^{2+}, Fe^{3+}). These interactions are important in soil fertility, soil genesis, and in suppressing the toxicity of metal ions in aqueous systems.

Fulvic acids are a heterogeneous mixture of weak, polymeric acids. Their acid properties may be examined by pH titration. The overall carboxyl acidity of a fulvic acid solution can be determined from the end point of an acid-base titration. The equivalent weights of the six fulvic acid samples studied in this work were discussed in Section 7.3.

Interpretation of the carboxyl acidity of fulvic acids has been attempted in terms of models involving discrete binding sites with unique protonation constants, or models involving a continuous distribution of acidic functional groups. A review of these models is given in Chapter 1. (Section 1.3.2).

In this chapter, fulvic acid titration data were

analysed by a non-linear least squares procedure in terms of a mixture of independent monoprotic acids.

8.2 EXPERIMENTAL

pH potentiometric titrations were performed on deoxygenated solutions of fulvic acid, citric acid or phthalic/malonic acid. Conductivity titrations for fulvic acid were performed in the absence of added electrolyte, whereas pH titrations were performed in 0.1 M KCℓ.

Ligand solutions were titrated with KOH (pH 3.7 - 8.7) and HCℓ (pH 3.7 - 2.8). Approximately 80 - 100 datum points were collected over the entire pH range for pH titrations. Experimental details are given in Chapters 3 and 4.

The total carboxyl acidity was determined from the KOH end point titre. The end point was determined by Grans analysis of the data in the pH range 8.7 - 7.9. For samples with high nitrogen contents (FA4, FAI, FAH; peat derived fulvic acids), the total carboxyl acidity was equated to the KOH titratable acidity plus the amino acid acidity. The amino acid acidity was determined from the nitrogen content, assuming that all nitrogen could be ascribed to amino acid groups. A nitrogen content of 2.3% equated to a maximum of 20% of carboxyl groups associated with amino acid residues. The zwitterionic amino acid groups were not titrated with KOH below pH 8, but were protonated below pH 3.5 during the HCℓ titration.

8.3 NUMERICAL ANALYSIS

The approach used in this study to interpret the carboxyl acidity of fulvic acids was to consider fulvic acid as a mixture of monoprotic acids.

The "titration constants" K_1', K_2', K_3' ... and stoichiometric concentrations of the monoprotic acids HA, HB, HC ... were determined using a least squares analysis of pH titration data. The numerical approach for this analysis was presented in Section 2.1.3.

The validity of this model was tested by analysis of titration curves for citric acid and for a mixture of malonic and phthalic acids.

8.4 RESULTS

(a) Citric acid

The result for analysis of a citric acid titration curve in terms of three monoprotic acids is given in Table 8.1, and is compared with the protonation constants reported in Chapter 5. Results are also given for fitting two and four acids to the titration curve.

The pH - dependent distribution curves for the protonation constant and titration constant models are shown in Figure 8.1.

(b) Malonic acid - phthalic acid mixture

The result for analysis of a mixture of malonic acid (64% of COOH) and phthalic acid (36% of COOH) is given in Table 8.2 and compared with the known pK values and composition. The synthetic mixture was considered as a mixture of three monoprotic acids.

Table 8.1 Citrate Protonation Constants and Titration Constants, 25°C, 0.1 M KCℓ

	Protonation Constants	Titration Constants		
		n=2	n=3	n=4
log K_1 (concentration, %)	5.70 ± 0.02	4.35 ± 0.76(49)	5.70 ± 0.01(33.5)	5.71 ± 0.14(33)
K_2	4.35 ± 0.01	3.15 ± 0.12(51)	4.34 ± 0.01(34)	4.39 ± 0.39(33)
K_3	2.91 ± 0.02		2.89 ± 0.01(32.5)	3.07 ± 1.10(23)
K_4				1.93 ± 1.97(11)
R-factor(%)	0.15	5.3	0.14	3.7

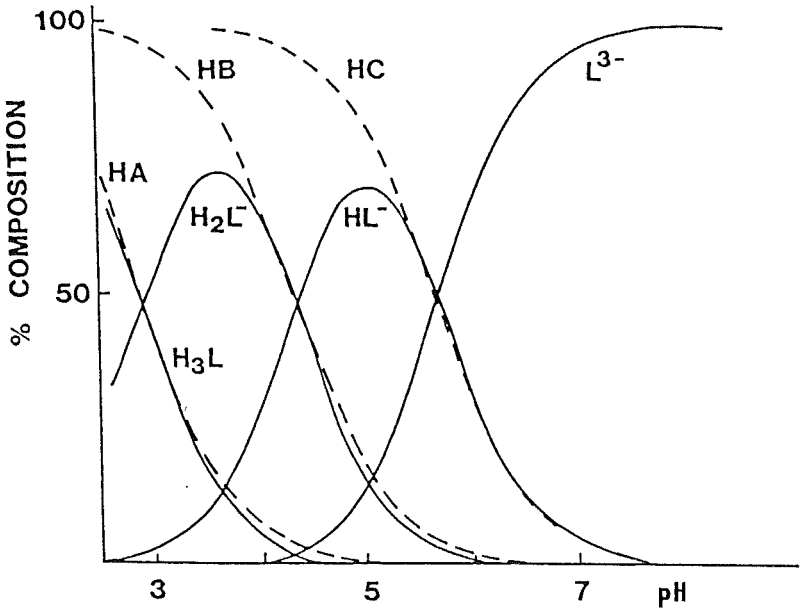


Figure 8.1 Computed solution composition for citrate-H^+ species as a function of $p[H^+]$. Citric acid considered as a tribasic acid ($[H_3L]=2.52\times10^{-3}$ M), —— ; considered as an equimolar mixture of moroprotic acids, --- . SEE ERRATA

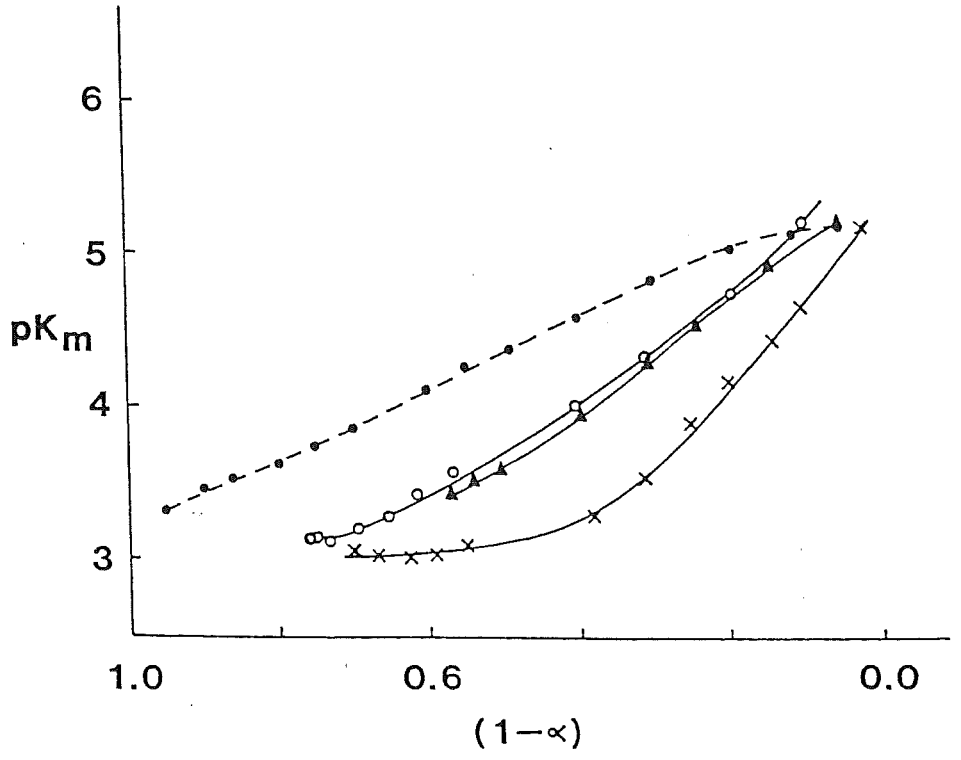


Figure 8.2 Plot of pK_m against degree of protonation $(1-\alpha)$ for citric acid, --- ; fulvic acid, ——— , FA1 (O), FA2 (▲), FA4 (x).

Table 8.2 Titration Constants for a Synthetic Malonate-Phthalate Solution, I=0.1 M KCℓ, 25°C

	Known values			Calculated values		
	(%)	log K_1	log K_2	(%)	log K_1'	log K_2'
malonate	32		2.61-2.87[b]	49.5		2.93
phthalate	18		2.72[a]			
phthalate	18	4.95[a]		18	4.92	
malonate	32	5.26-5.69[b]		32.5	5.50	

a Kennedy et al. (1983)

b Perrin (1979)

(c) Fulvic acid

During the computation of "titration constants", SEE ERRATA values for α (degree of deprotonation) and pK_m (= pH - nlog $\alpha/1-\alpha$) are calcualted for each datum point. pH titration data were first plotted as pK_m vs. $1-\alpha$ (Figure 8.2) to ensure that the acid and alkali titration data generated a continuous curve. Datum points in the overlap region were removed from the data set.

The least squares analysis was attempted in terms of two, three, four and five monoprotic acids. Models containing two and five acids would not refine. The best numerical fit was obtained for four acids. The results for six fulvic acid samples are given in Table 8.3. For one sample (FA2), the result for interpretation as three acids is given for comparison.

A conductivity titration was performed on one fulvic

Table 8.3 Titration Constants and Concentrations (%) for Fulvic Acids, I = 0.1 M KCℓ, 25°C

	$\log K_1'$	C_1	$\log K_2'$	C_2	$\log K_3'$	C_3	$\log K_4'$	C_4	R-factor(%)
FA1	6.67 ± 0.02	9	5.53 ± 0.06	11	4.45 ± 0.06	27	2.73 ± 0.03	51	3.5
FA2	6.59 ± 0.03	10	5.33 ± 0.06	16	4.39 ± 0.08	20	2.73 ± 0.05	54	1.0
	6.32 ± 0.07[a]	15	4.65 ± 0.07	30	2.68 ± 0.04	55			2.5
FA4	6.50 ± 0.08	6	5.57 ± 0.17	7	4.67 ± 0.04	18	2.63 ± 0.02	70	1.3
FAI	7.19 ± 0.02	7	5.91 ± 0.03	10	4.82 ± 0.01	23	3.02 ± 0.02	60	1.3
FAH	7.03 ± 0.05	5	5.61 ± 0.04	9	5.03 ± 0.04	10	3.20 ± 0.02	76	1.0
FAS	6.70 ± 0.02	9	5.44 ± 0.04	17	4.08 ± 0.04	26	2.44 ± 0.02	48	1.0

a Considered as 3 monoprotic acids.

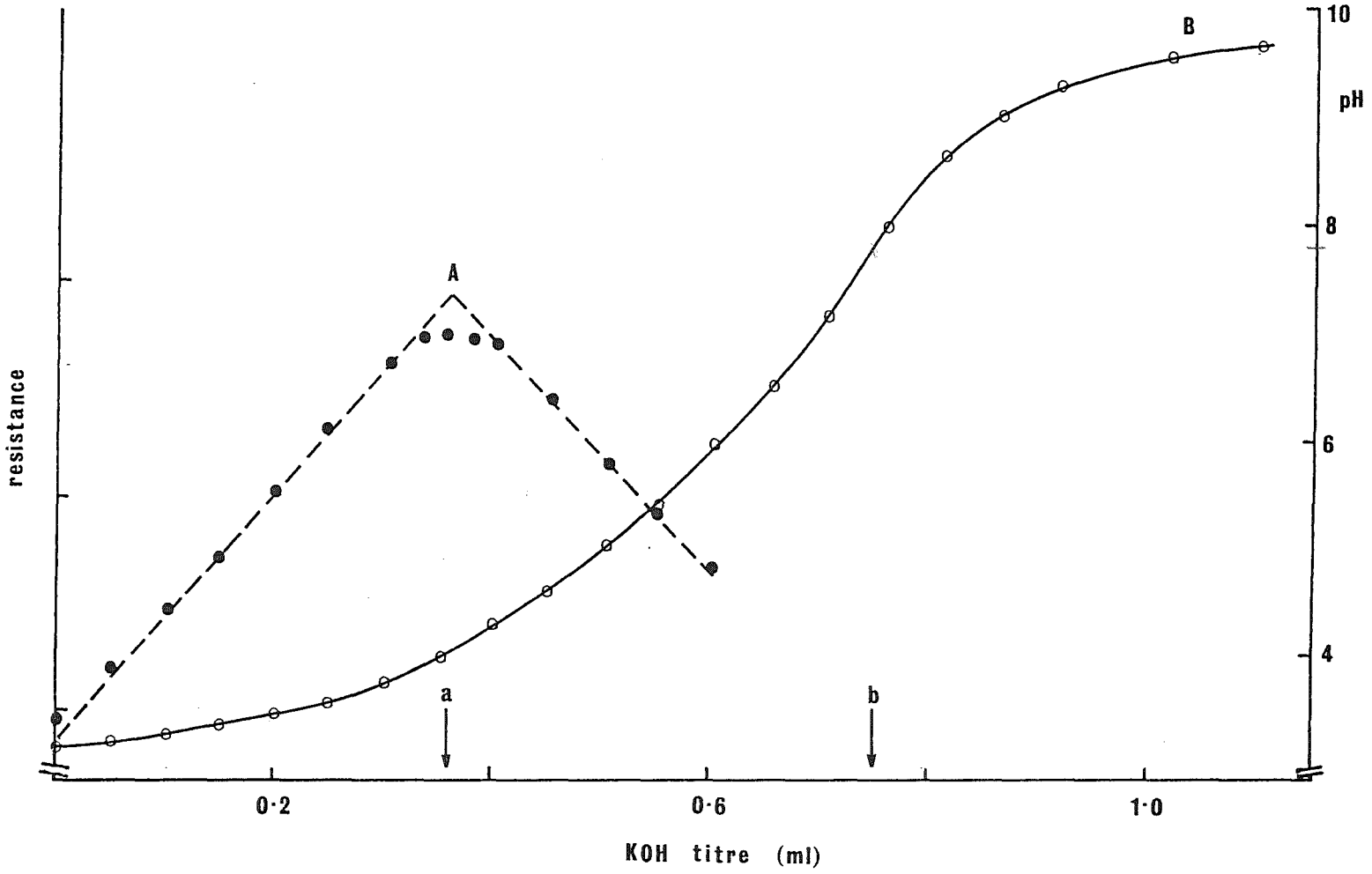


Figure 8.3 Conductivity (A) and pH (B) curves for titration of fulvic acid (FA1, 82.5 mgℓ^{-1}) with standard KOH (0.077 M). Conductivity end point, a = 0.360 mℓ ; pH end point, b = 0.750 mℓ.

acid (FA1). It showed a distinct end point (maximum) at 48% of the alkali titre required to reach the pH titration end point and is shown in Figure 8.3.

8.5 DISCUSSION

8.5.1 *The Titration Curve*

The fulvic acid titration curve is featureless and has a poorly defined end point. The curve shows a gradual rise in pH with added alkali, indicating buffering over a wide pH range and the diverse nature of carboxyl environments. The curve rises less steeply (i.e. there is more buffering) than a malonic acid or citric acid titration curve, suggesting that fulvic acid contains more than three general types of functional groups with differing acidities.

An often encountered problem in fulvic acid titrations is drifting pH. In this study all samples were filtered through a 0.025 μ membrane to remove colloidal material, either during the acid pyrophosphate/XAD-7 extraction or (for FAS) following dissolution in Milli-Q deionised water.

Severe pH drifting was observed for FAS prior to filtration through a 0.025 μ membrane, but no drifting was observed after filtration. All acid pyrophosphate/XAD-7 extracted fulvic acids were titrated without noticeable pH drifting, except for FA3 (B_s horizon sample). The titration of FA3 suffered from severe pH drifting probably because of its high ash content (Table 7.1). Similarly, the fulvic acid sample FAA (acid extracted) had a high ash content (Table 3.1) and showed severe pH drifting. Titration data for FA3 and FAA were not considered for numerical analysis.

Titration up to pH 8.7 did not result in hysteresis,

i.e. the reverse titration curve was coincident with the forward titration. However, a difference between the forward and reverse titration curves was observed when the forward titration was continued up to pH 9.7. The forward and reverse titration curves then coincided at both ends, but towards the end point the curves separated; the pH of the reverse titration was lower than that for the forward titration for equivalent amounts of titre. More acidic groups were reprotonated in the reverse titration than were dissociated in the forward titration.

Since this titration was performed in an inert nitrogen atmosphere, the result suggested that non-oxidative reactions were induced at high pH. Saponification of ester groups at high pH would result in the generation of acidic functional groups which would be titrated by H^+ during the reverse titration but not titrated during the initial forward titration. Alkaline hydrolysis of fulvic acid was discussed in Section 7.5.

Davis and Mott (1981a, b) have observed hysteresis in fulvic acid titrations. They ascribe this phenomenon to alkali consuming reactions of polysaccharides. However, in this work, IR spectra (Figure 7.1) indicated that the fulvic acid samples contained very little polysaccharide material.

Paxeus and Wedborg (1985) have also shown that fulvic acid exhibits hysteresis following titration above pH 8.

8.5.2 *Citrate Titration Constants*

The resutls in Table 8.1 indicate that for the polyprotic acid citric acid, interpretation of the titration curve as three independent monoprotic acids provides an

equally acceptable numerical fit.

In contrast to citric acid, the number of independent acid types for fulvic acid is unknown. Therefore, to apply this numerical approach to fulvic acid titration data, it was necessary to establish that the best numerical fit for citric acid was obtained when it was considered as three acids. This can be seen by comparing the results for models assuming two, three and four monoprotic acids (Table 8.1). Both the uncertainties associated with each constant and the R-factor are considerably improved when the "correct" number of acids is considered.

8.5.3 *Malonate-Phthalate Mixture Titration Constants*

The results in Table 8.2 indicate that for the mixture of polyprotic acids, malonic (64% COOH) and phthalic (36% COOH), the derived titration constants are in close agreement with the protonation constants for the individual acids.

Log K_2 values for the individual acids cannot be distinguished, and were considered as one averaged constant.

In contrast to citrate, this mixture of ligands has protonation constants with log K < 1.0 (log K_1 phthalate 4.95, malonate 5.5). Analysis of these data highlighted the problems faced in differentiating functional groups having similar protonation constants.

Initially, log K_1' for malonate and phthalate were not differentiated; only a single mean constant and total concentration resulted from the least squares analysis (log K_1' 5.29, 51% of carboxyl groups). However, if log K_1' was fixed at 5.5, or if the concentration of more basic malonate

carboxyl groups was fixed at 32% (i.e. the known value), then a satisfactory fit was obtained in terms of three monoprotic acids (see Table 8.2).

This approach, of arbitrarily fixing a constant or concentration at a predetermined value, is satisfactory for systems where the constants or number of constants are known, but for fulvic acid all constants and concentrations are unknown.

If data in the pH range 4.9 to 5.7 were deleted, i.e. from a region where there is considerable overlap of malonate and phthalate equilibria, then a satisfactory fit was obtained for all parameters. However, once again, this approach required a knowledge of the values for the overlapping constants so that the appropriate data could be deleted.

A method was sought in which the value of the extreme constant (log K_1' or log K_n') or concentration could be determined from a portion of the data set, then included as a fixed value for solution of the complete data set.

Log K_1' and the associated concentration for malonate were determined by analysis of data specifically relating to the protonation of malonate, i.e. pH > 5.7. In this least squares calculation, any reasonable value could be assumed for log K_2' (assuming log K_2' = 3.7, for the remaining acidic groups, they would contribute less than 1% to the group being protonated above pH 5.7). The concentration of carboxyl groups associated with log K_2' was defined as: total ligand - concentration of K_1' acidic groups. Then, with either log K_1' or its associated concentration fixed, all data were subjected to least squares analysis. A satisfactory fit was obtained in terms of three monoprotic acids (Table 8.2).

When the data were interpreted in terms of four monoprotic acids, the additional parameter was rejected.

The same numerical approach can be used to define log K_n' (the strongest acidic group), by considering low pH data.

An equivalent graphical approach has been used by Bolles and Drago (1965) for simultaneous determination of K and one other intensive or extensive property of a single equilibrium reaction.

8.5.4 *Fulvate Titration Constants*

Fulvic acid titration curves were satisfactorily described by a mixture of four monoprotic acids (Table 8.3). The titration constants fell within four regions; log K_1' 7.2 -6.5, log K_2' 5.9 - 5.3, log K_3' 5.0 - 4.1 and log K_4' 3.2 - 2.4.

Paxeus and Wedborg (1985) reported a similar interpretation of soil fulvic acid titration data. Least squares analysis in terms of n-monoprotic acids indicated four monoprotic carboxylic acids (and two phenolic acids); log K_4' 2.75 (48%), log K_3' 4.38 (28%), log K_2' 5.59 (16%), log K_1' 6.85 (9%).

Erble and Feuerstein (1979) reported a "discrete pK spectrum" for humic acid. Using fixed pK values at intervals of $\underline{c}$ 0.5 pK units, the pK vs. concentration spectrum showed six concentration maxima. Four of these maxima were similar to those calculated in this study; log K_4' 2.85 (47%), log K_3' 4.30 (25%), log K_2' 5.40 (17%), log K_1' 6.50 (11%). The behaviour of humic acid was in accord with the presence of chemically independent monoprotic acids.

These constants fall within distinct groupings of

log K values observed for a wide range of mono- and polyprotic carboxylate groups :

(a) log K > 5.7 : log K_1 for n-protic aliphatic acids (n > 3); dialkyl malonates.

(b) log K 5.7 - 4.9 : log K_1 for diprotic and triprotic aliphatic acids (e.g. malonic, citric).

(c) log K 4.7 - 4.2 : log K_2 for triprotic aliphatic acids, aliphatic and aromatic monoprotic acids (e.g. acetic, benzoic); log K_1 for m- and p- hydroxybenzoic acids.

(d) log K 2.8 - 2.4 : log K_2 for diprotic aliphatic acids; log K_3 for triprotic aliphatic acids; log K_2 for salicylic acids; log K for α-amino acids.

Examination of the "titration" constants for fulvic acids presented in Table 8.3 reveals that the separation between successive log K_n' values is close to 1.0, i.e. log $K_1'/K_2' \leq 1.0$ and log $K_2'/K_3' \leq 1.0$. The possibility that pairs of constants with Δ log K < 1.0 were concealed, as occurred in the phthalate/malonate system (Section 8.5.3), was considered.

The extrema log K values and concentrations were calculated from portions of the data set prior to the least squares analysis of the complete data set. Log K_1' was calculated from high pH data (pH > 6), with log K_2' fixed at an arbitrary value of 3.0. Similarly, log K_n' was calculated from low pH data (pH < 3), with log K_1' fixed at 6.5.

With one or both extreme fixed, the complete data set was then analysed by the least squares procedure. The titration data were best described by four monoprotic acids; there was no evidence for a fifth constant. Further, analysis of titration data by this method gave an identical set of constants to that obtained from a general least squares analysis.

Interpretation of Titration Constants

The fulvic acids can be divided into two groups; those extracted by the acid pyrophosphate/XAD-7 method (FA1, FA2, FA4) and those exposed to alkali (FAI, FAH, FAS). In general, the constants (log K) for alkali exposed samples are slightly higher (0.2 to 0.4 log units). The effect of alkali exposure on titration constants was discussed in Section 7.5.3.

A striking feature of the distribution of acidic groups is the high proportion (50 - 70%) with a weighted mean log K_4' in the range 3.2 - 2.4. This high proportion of the most acidic groups will account for the solubility of fulvic acid and the low pH of such solutions (pH $\leq$ 3.7 for 75 mg ℓ^{-1}). These groups will be substantially dissociated in dilute aqueous solution, and study of their protonation reactions requires titration with a strong acid.

The high proportion of acidic groups with log K_4' in the range 3.2 to 2.4 would facilitate considerable carboxylate-carboxyl and carboxylate-phenolic inter- and intramolecular hydrogen bonding. Wershaw (1986) postulates these types of interactions in his "aggregation" model for fulvic acids.

A value greater than 5.7 for log K_1' can only arise if polyprotic acids (n > 3) or dialkyl malonates are present. It is calculated that not more than 5 - 10% of carboxyl groups

in fulvic acid are due to such acid moieties. Log K_2' would include the 5 - 10% of carboxyl groups in polyprotic acids ($n > 3$) undergoing their second protonation (log K_2). It is inferred that the remaining 5 - 10% of carboxyl groups in this bracket is due to polyprotic acids with $4 > n \geq 2$, e.g. K_1 for citrate or malonate.

The low proportion of carboxyl groups relating to polyprotic (polydentate) acid moieties implies that a high proportion of the groups contributing to log K_4' $\leq$ 2.7 belong to isolated monocarboxyl entities such as salicylates or amino acids.

8.5.5 *Conductivity Titrations*

The conductivity end point of a titration determines the end point for titration of the strong acid component. The conductivity end point for FA1 (48% of total carboxyl end point) was consistent with the calculated proportion of most strongly acidic carboxyl groups, log K_4' 51% (Table 8.3). Similarly, there was a correlation between the conductivity end point for FAS (50%; Cressey et al., 1983) and its most acidic fraction (log K_4' 50%).

Gamble (1970) observed a conductivity end point at $\leq$ 40% of the equivalence point for total carboxyl groups. By comparison of calculated K_a values for these "type I" carboxyl groups with literature K_a values, Gamble concluded that carboxyl groups ortho to phenolic -OH groups were responsible for the conductivity end point. However, it is also noted that some polyprotic acids have pK values in the same region, e.g. citric, malonic, phthalic acids, as do amino acids.

8.5.6 *pK_m vs. α Plots*

Figure 8.2 is a plot of pK_m vs. (1-α) for fulvic acids and for citric acid. This plot shows minimal variation in pK_m for (1-α) values between 1.0 and 0.6, i.e. the slope is approximately zero. This is in contrast to the plot for a polyprotic acid such as citric acid. It suggests that a high proportion of carboxyl groups are not associated with polyprotic entities, but rather are present as isolated monoprotic or diprotic entities (slope $\underline{c}$ 0; Young et al., 1981).

The high proportion of isolated acidic groups is not consistent with a polyelectrolyte model for fulvic acids.

CHAPTER 9

METAL-FULVIC ACID COMPLEXING : A COMPARATIVE STUDY

9.1 INTRODUCTION

The topic of metal complexation by humic substances is of considerable importance. Metal-humate complexing may affect the transport of metals through natural systems (e.g. soils and waters), and may also affect the bioavailability of metals.

Quantifying the role of humic substances in metal complexing has proven difficult. Although all fulvic acids may contain carboxyl, phenolic, alcoholic and amino groups, the proportions and arrangement of these groups within a macromolecule may vary from sample to sample. Further, the analytical methods used to measure these interactions are often inadequate. For example, many methods suffer from lack of sensitivity and specificity. For further details, see Section 1.4.2.

The common parameters used to define the complexing ability of fulvic acid are conditional stability constants and the binding capacities. However, because of the presence of a variety of functional groups of differing binding strengths, these parameters will vary with pH and ionic strength. It becomes difficult to compare values from different studies. Further, the value obtained for the stability constant will be dependent on the model employed for numerical analysis.

This study made no attempt to calculate conditional stability constants for fulvic acid - metal complexes. An alternative approach was adopted in which the complexing ability of fulvic acids from a variety of sources was compared to that for simpler ligands (e.g. citric acid). Inferences concerning binding sites and strengths for fulvic acids were made from these comparative studies.

Techniques including ion selective electrode potentiometry (ISE), fluorescence quenching (FQ) and polarography were used. The results are discussed in Sections 9.2, 9.3 and 9.4 respectively.

9.2 ION SELECTIVE ELECTRODE STUDIES

ISE potentiometry was used to monitor the free metal concentration of copper(II)-ligand solutions as a function of pH. A ligand concentration of 1.8×10^{-4} M (carboxyl group concentration) was used; this corresponds to 10 -20 mg/ ℓ fulvic acid, a realistic concentration in natural waters. A realistic Cu(II) concentration in natural waters would be $\underline{c}\ 10^{-7}$ to 10^{-8} M (Florence & Batley, 1977). However, the limit of detection of the ISE is $\underline{c}\ 10^{-7}$ M, as indicated by the linear response range of the copper(II) ISE used in this study (pM 2.7 to 7.5). Thus, for this technique, the minimum total Cu(II) concentration used was 9.0×10^{-6} M (1:20 Cu(II):COOH). This concentration allowed 1% free metal ion in solution (9.0×10^{-8} M to be detected with some certainty. Titrations were also performed at a higher Cu(II) concentration, 4.0×10^{-5} M (1:4.5 Cu(II):COOH). Experimental details are described in Chapters 3 and 4.

SEE ERRATA

9.2.1 *Results*

The % free Cu(II) vs. pH curves for 1:20 and 1:4.5 solutions are shown in Figure 9.1, for fulvic acids FA1 (B_h), FA2 (B_h), FA4 (peat) and FAI (peat, alkali extracted). The curves were determined for equal concentrations of carboxyl group for each fulvic acid at both ratios; the Cu(II) concentration was varied.

The fulvic acids show similar abilities to bind Cu(II) at a given Cu(II):COOH ratio. The difference in % free Cu(II) at any given pH is at most 10 - 15%. The binding curves at higher loading of copper are displaced to higher pH.

Figure 9.2 compares the binding curves for fulvic acid (presented as a band) with those for model ligands (aspartic, citric, malonic, salicylic acids) at the same carboxyl concentration, and Cu:COOH 1:20 (Figure 9.2a) and 1:4.5 (Figure 9.2b). The curves for the model ligands at 1:4.5 were determined by experiment and compared to those computed (see Section 2.6.2) from known stability constants. The equilibrium models used to compute the binding curves were:

	Citrate	Aspartate	Malonate	Salicylate
$L + H \Leftrightarrow HL$	5.70[a]	9.71[c]	5.26[d]	13.6[d]
$L + 2H \Leftrightarrow H_2L$	10.06	13.42	7.96	16.4
$L + 3H \Leftrightarrow H_3L$	12.96	15.12		
$Cu+L \Leftrightarrow CuL$	6.03[b]	8.84	5.09	10.80
$Cu+H+L \Leftrightarrow CuHL$		12.70		15.24
$Cu+2L \Leftrightarrow CuL_2$	10.43	15.24	7.63	17.80

[a] (Gregor & Powell, 1986b)
[b] (Ramamoorthy et al., 1972)
[c] (Claridge et al., 1980)
[d] (Perrin, 1979)

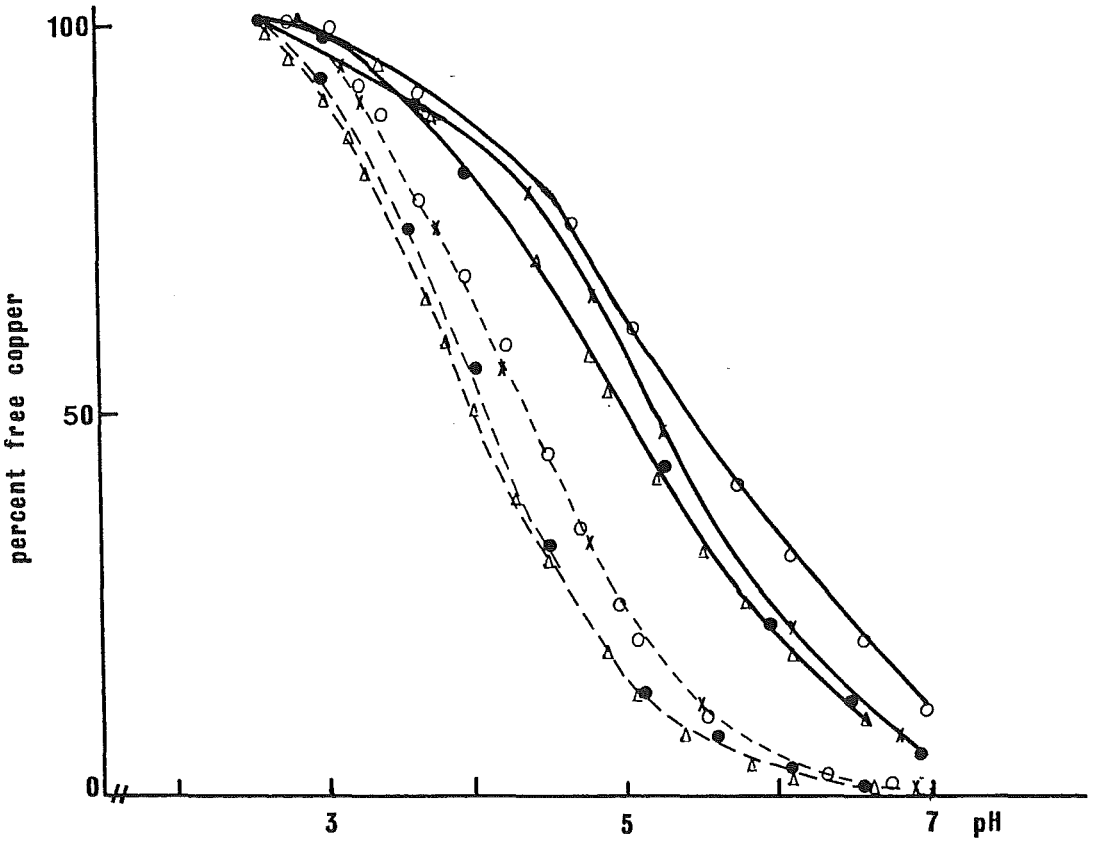


Figure 9.1 Copper(II) binding curves for fulvic acids (1.8×10^{-4} M COOH),
Cu(II) : COOH 1:20 (---), 1:4.5 (——) .
Δ, FA1 (B_h) ; x, FA2 (B_h) ; O, FA4 (peat) ;
●, FAI (IHSS).

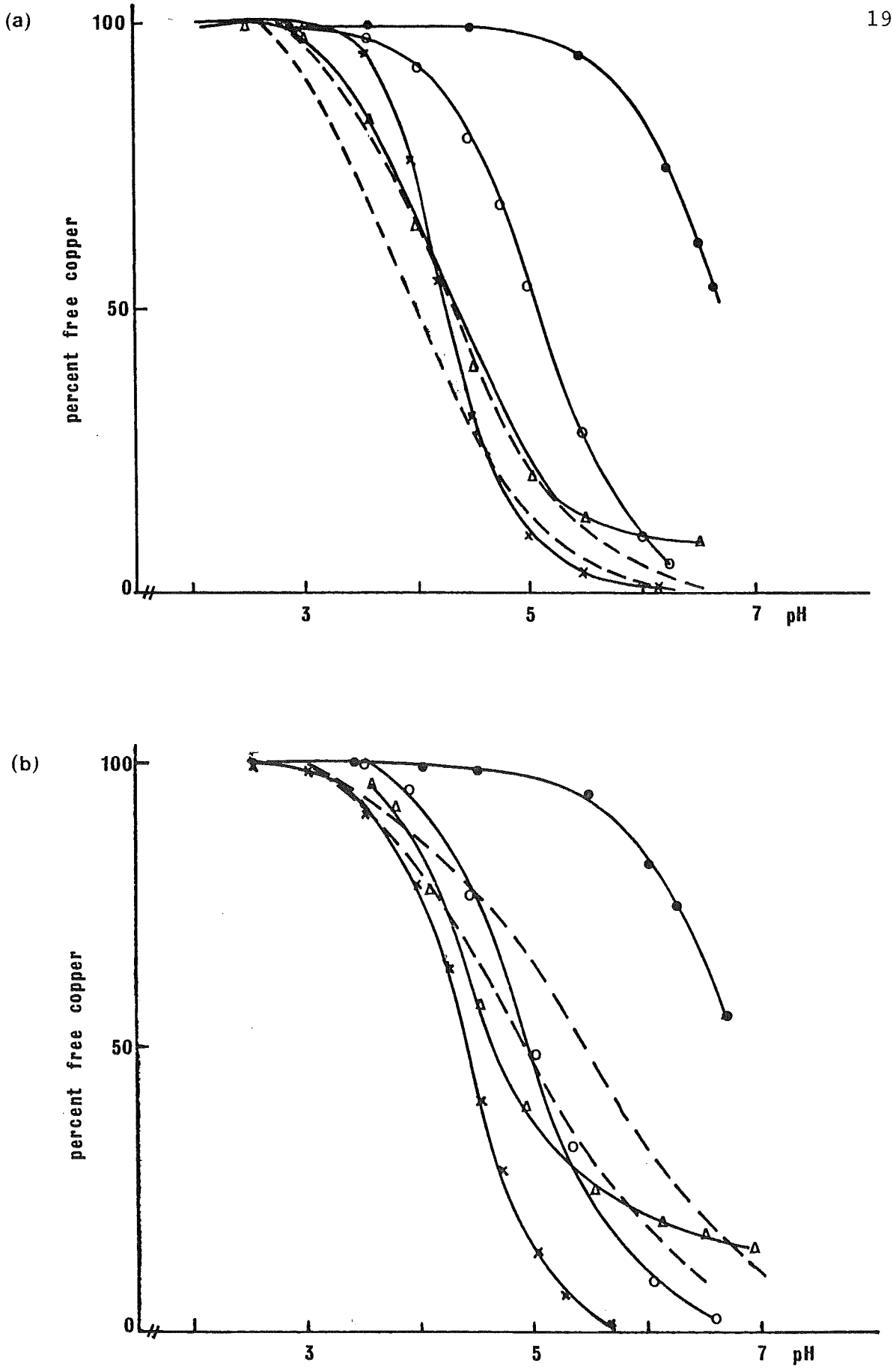


Figure 9.2 Copper(II) binding curves for aspartic (o), citric (x), malonic (Δ) and salicylic (●) acids (1.8×10^{-4} M COOH).
(a) Cu(II):COOH 1:20, computed curve.
(b) Cu(II):COOH 1:4.5.
Fulvic acid binding curves lie between dashed lines.

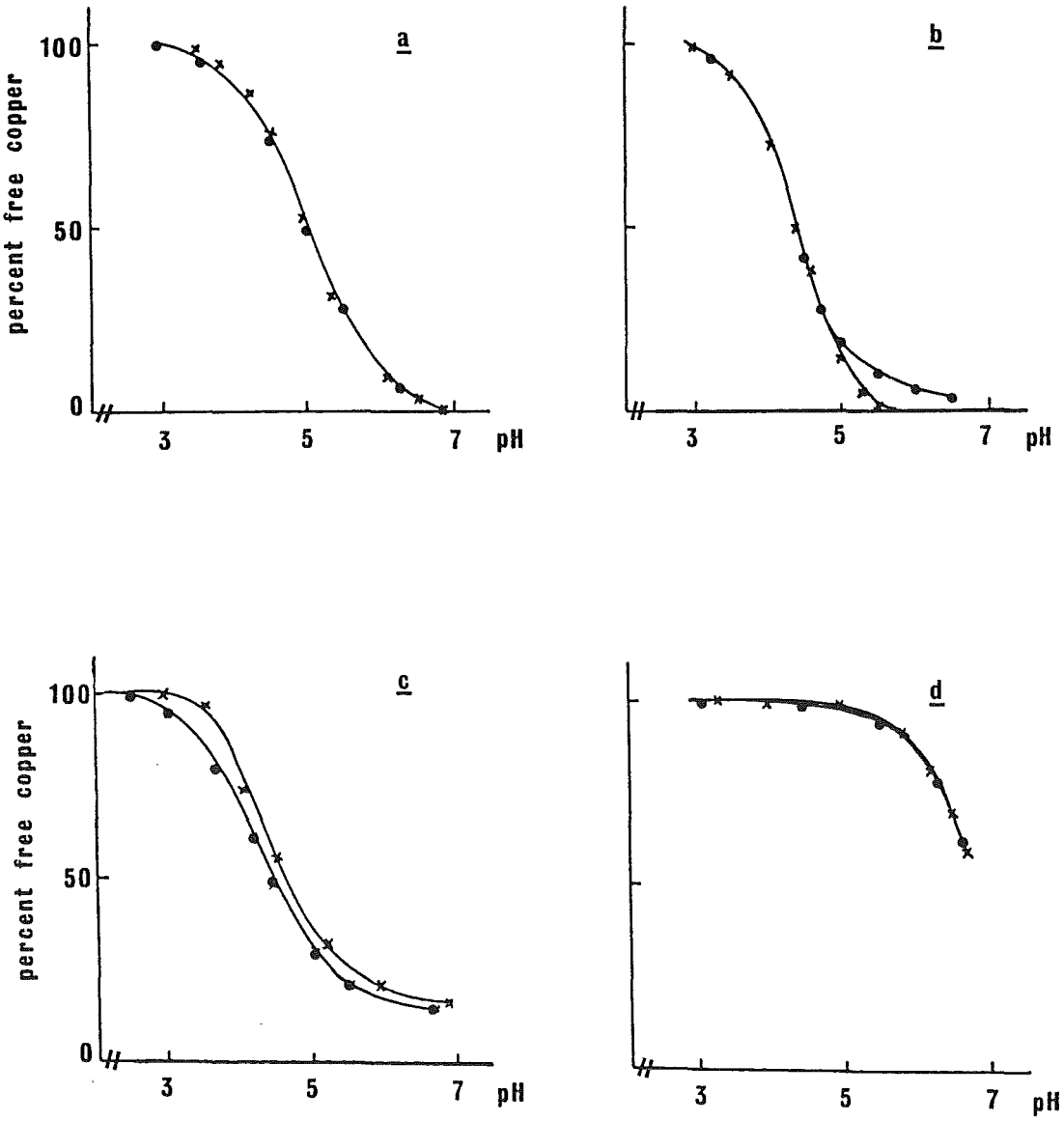


Figure 9.3 Copper(II) binding curves for aspartic (a), citric (b), malonic (c) and salicylic (d) acids (1.8×10^{-4} M COOH, Cu(II): COOH 1:4.5), as determined by ion selective electrode (x) or computer modelling (●).

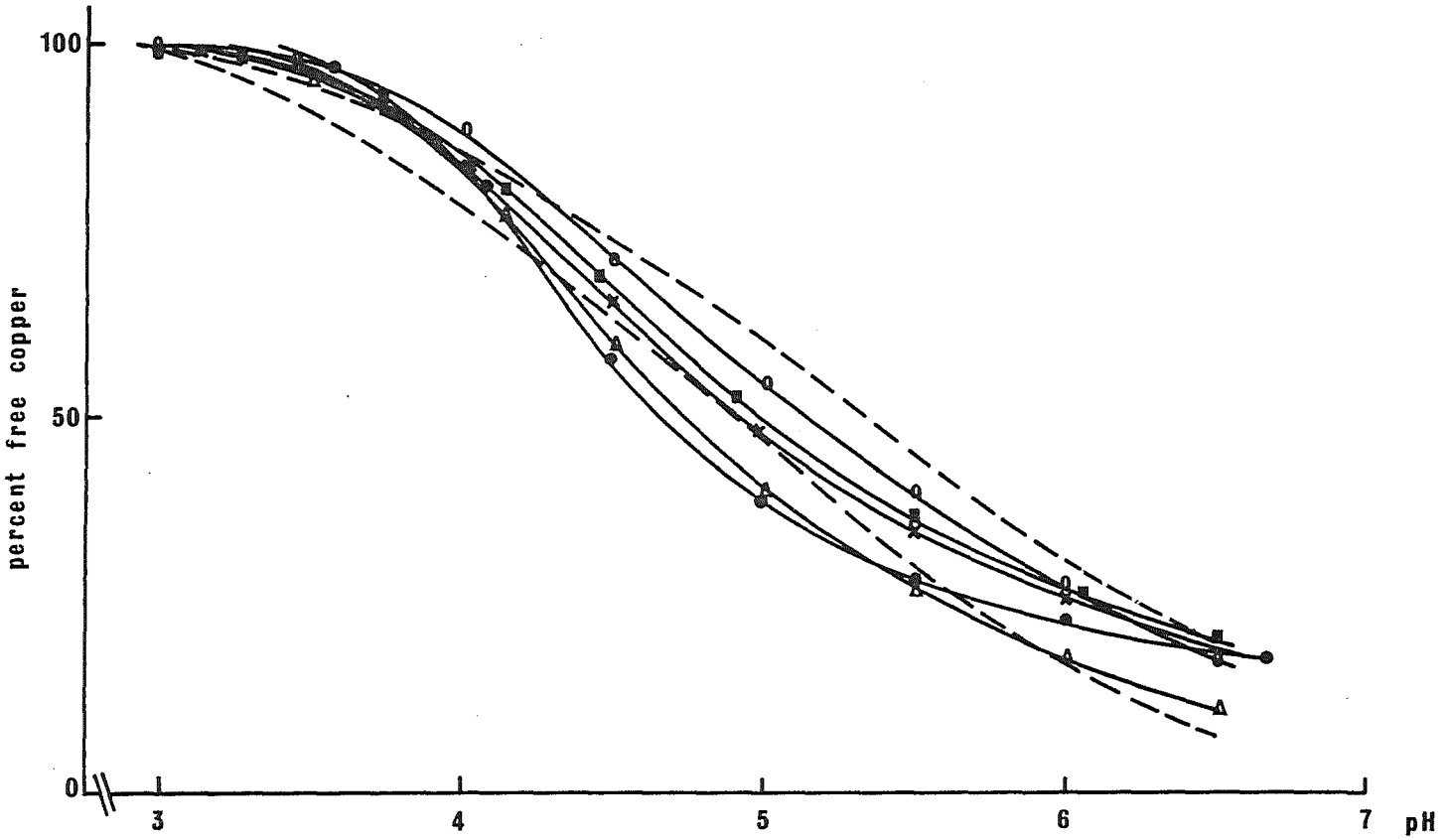


Figure 9.4 Computed copper(II) binding curves for competing ligand models (1.8×10^{-4} M COOH, Cu(II):COOH 1:4.5). Model (i), Δ ; (ii), 0 ; (iii), x ; (iv), ● ; (v), ■ . Refer to text for compositions of models. Fulvic acid binding curves lie between dashed lines.

The agreement between experimentally derived and calculated binding curves was good (Figure 9.3).

These equilibrium models were used to predict binding curves for the model ligands at 1:20. The curves were not determined experimentally at this ratio.

As can be seen from Figure 9.2, fulvic acid binding curves do not resemble any one model acid over the entire pH range of complexing.

Binding curves were computed (see Section 2.6.2) for mixtures of model ligands in an attempt to mimic the fulvic acid binding curves. The results for several synthetic mixtures (1.8×10^{-4} M COOH, 4.0×10^{-5} M Cu(II)) are shown in Figure 9.4.

9.2.2 *Discussion*

A commonly encountered problem in ISE studies is the effect of ligand adsorption upon the response of the electrode. Adsorption of organic matter may result in sluggish response, and to shifting potentials relative to electrodes free from adsorption (Durst, 1978).

Effects of ligand adsorption were observed in this study. The presence of ligand (at a pH where complexing was not occurring) had the effect of shifting emf readings to less negative values ($\underline{c}$ 4 - 7 mV) relative to solutions in the absence of ligand. Consequently, the ISE was calibrated separately for each ligand; standard Cu(II) solutions were prepared in acidified (pH $\underline{c}$ 2.5) ligand solutions (see Chapter 4).

In Figure 9.1 the % free Cu(II) is plotted as a function of pH. Complexing commenced at pH 3 for the 1:4.5

solutions, and at pH 2.7 for the 1:20 solutions. The Cu(II) was 90% complexed at pH 7 for the 1:4.5 solutions and at pH 5.5 for the 1:20 solutions.

At either ratio of Cu(II):COOH, the curves for the 4 fulvic acid samples were separated by $\underline{c}$ 0.4 pH for a given % free Cu(II). This indicates that the samples vary in the stabilities of their complexes formed with Cu(II). The curves displaced to higher pH indicate less stable complexing.

The relative binding strengths of the alkali extracted IHSS peat fulvic acid (FAI) and the fulvic acid extracted from the same peat sample by the pyrophosphate/XAD-7 method (FA4) are compared in Figure 9.1. The binding curve for FAI is displaced to lower pH by $\underline{c}$ 0.4 pH. This indicates that the FAI fulvic acid sample (alkali extracted) forms more stable complexes with Cu(II) than does the mildly extracted FA4 sample. The shift to lower pH is consistent with an increase in carboxyl group density (cf. malonic and citric acids, Figure 9.2b), as may arise from ester hydrolysis during extraction with alkali. This inference is supported by the acid-base properties of the two samples (Chapters 7 and 8).

The curves for all fulvic acids were displaced to higher pH at the higher Cu(II) concentration (1:4.5), i.e. there was a larger % free Cu(II) at each pH. At 50% complexed Cu(II), the 1:45 curves were displaced by 1.0, 0.85, 1.0 and 0.95 pH for FA1, FA2, FA4 and FAI respectively. A much smaller displacement was observed for the model ligands ($\underline{c}$ 0.1 pH; Figure 9.2). The greater displacement for fulvic acids implies that there is a variety of groups involved

in complexing. The groups will have different affinities for metal ions. The most strongly binding sites will be involved in complexing initially, followed by increasingly weaker binding sites at higher concentrations of metal. Thus at low Cu(II) concentration (1:20) the fulvic acid binding curves more closely resemble the binding curve for citric acid, forming the most stable of the Cu(II)-ligand complexes studied (Figure 9.2a). At higher Cu(II) concentrations (1:4.5), the fulvic acid binding curves are more similar to that for aspartic acid which forms less stable Cu(II) complexes than does citric acid (Figure 9.2b).

Cressey et al. (1983) observed similar results for Cu(II) complexing by an alkali extracted fulvic acid and an acid extracted fulvic acid, at [COOH] = 1.6×10^{-3} M and Cu(II):COOH ratios of 1:4 or 1:2.

As can be seen in Figure 9.2, no single model ligand can adequately describe Cu(II) - fulvic acid complexing over the entire pH range of complexing. Further, a variety of complexing groups with differing metal affinities is indicated by the substantial shift in binding curves with changes in metal loading. The copper(II) binding curves for fulvic acid were approximated by considering mixtures of competing ligands (acetic, aspartic, citric, malonic, salicylic and pentane-3,3-dicarboxylic acids). The results for five computer-generated binding curves are presented in Figure 9.4. All models and fulvic acids were considered at the same total COOH concentration, 1.8×10^{-4} M. No allowance was made for possible mixed-ligand complexes. The five model systems were:

(i) aspartic (10%), salicylic (22%), malonic (20%), citric (36%), pentane-3,3-dicarboxylic (12%).

(ii) aspartic (10%), salicylic (32%), acetic (10%), citric (36%), pentane-3,3-dicarboxylic (12%).

(iii) malonic (55%), citric (25%), aspartic (20%).

(iv) citric (30%), salicylic (50%), acetic (20%).

(v) citric (25%), malonic (40%), acetic (15%), aspartic (20%).

Of these five models, three describe Cu(II) - fulvic acid complexing particularly well; they were models (ii), (iii) and (v).

The pH-dependent distribution curves for individual ligands indicate that not all groups are involved in binding copper. The pH-dependent distribution curves for models (ii) and (v) are shown in Figure 9.5. These distribution curves clearly show that in a mixture of complexing groups with differing metal affinities, the groups involved in complexing may change with changing pH. Those complexing groups that form the weaker complexes (e.g. acetic, salicylic, pentane-3,3-dicarboxylic acids) only become important at high pH or, in the case of acetic acid, not at all.

The mixtures of ligands that were found to best approximate fulvic acid complexing were not without chemical relevance to the known composition of the fulvic acids. For example, the proportion of aspartic acid considered in the model was never greater than 20%. This limit to the contribution by amino acid structures was based on the maximum

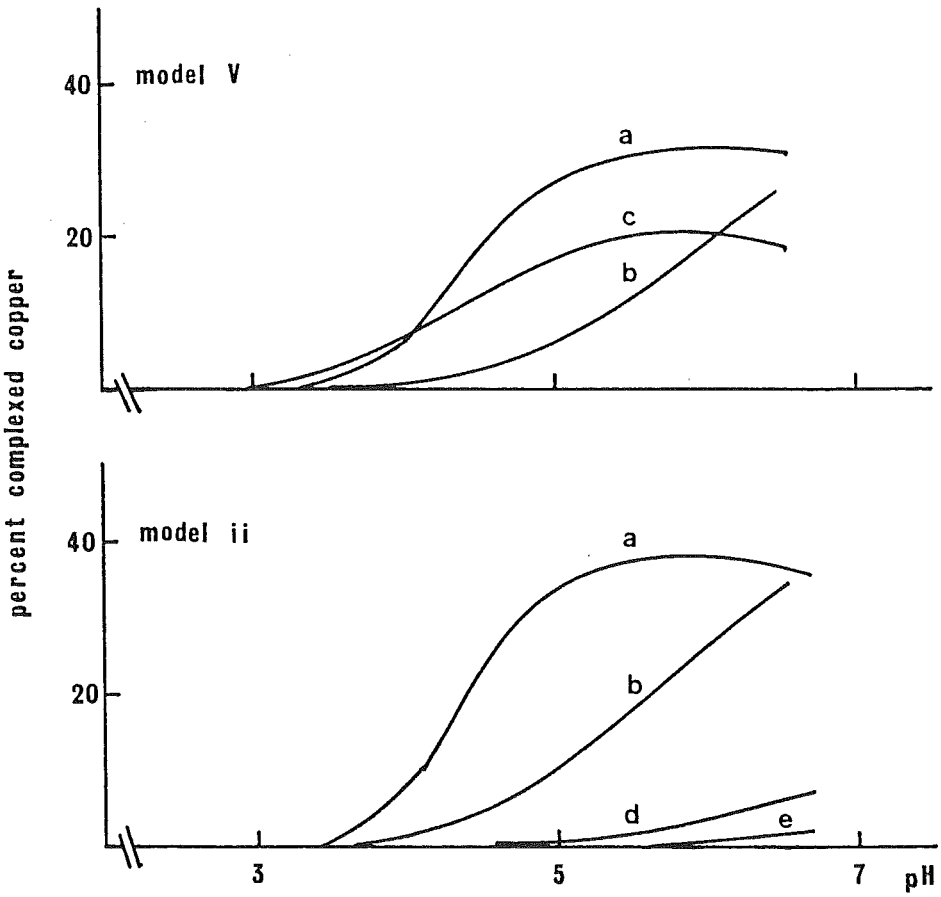


Figure 9.5 Computed solution compositions for copper(II) - competing ligand models (ii) and (v). Cu(II)-citrate (a), Cu(II)-aspartate (b), Cu(II)-malonate (c), Cu(II)-pentane-3, 3-dicarboxylic acid (d), Cu(II)-salicylate (e).

nitrogen content of the fulvic acid samples (2.3%).

Interestingly, these models can be related to the protonation constants and respective concentrations for the fulvic acid samples, as discussed in Chapter 8. The carboxyl group acidity of fulvic acid was best described by 4 (weighted mean) protonation constants. These were: $\log K_4' \underline{c} 2.7$ ($\underline{c}$ 60% of the total carboxyl groups), $\log K_3' \underline{c} 4.6$ (20%), $\log K_2' \underline{c} 5.6$ (12%), and $\log K_1' \underline{c} 6.8$ (6%). As was discussed in Chapter 8, these 4 weighted values of protonation constants coincide with the groupings of constants for simple aliphatic and aromatic carboxylic acids. It is possible to combine several of these simple carboxylic acids in appropriate proportions to approximate the log K distribution of fulvic acid. For example,

$\log K_1'$ - aliphatic tetracarboxylic acid, K_1 (6%)

$\log K_2'$ - aliphatic tetracarboxylic acid, K_2 (6%)
aliphatic tricarboxylic acid, K_1 (6%)

$\log K_3'$ - aliphatic tetracarboxylic acid, K_3 (6%)
aliphatic tricarboxylic acid, K_2 (6%)
aliphatic dicarboxylic acid, K_1 (10%)

$\log K_4'$ - aliphatic tetracarboxylic acid, K_4 (6%)
aliphatic tricarboxylic acid, K_3 (6%)
aliphatic dicarboxylic acid, K_2 (10%)
amino acid, K_1 } (38%)
o-hydroxybenzoic acid, K_2 }

A variation of this combination would be to replace the dicarboxylic acid (K_1) with a monoprotic acid (K_1), and adjust the amino acid/o-hydroxybenzoic acid contribution to account for the loss of dicarboxylic acid (K_2).

This type of distribution of acidic functional groups can then be used to calculate a Cu(II) binding curve, for comparison with the fulvic acid binding curves. However, for this specific example, it was not possible to calculate the binding curve because the protonation constants and Cu(II) stability constants for a tetracarboxylic acid are not known.

A further combination of simple acids was found to approximate fulvic acid carboxyl acidity; in this case all protonation constants and Cu(II) stability constants were available in the literature.

log K_1' -	dialkyl malonic acid, K_1	(6%)
log K_2' -	aliphatic tricarboxylic acid, K_1	(12%)
log K_3' -	aliphatic tricarboxylic acid, K_2	(12%)
	aliphatic dicarboxylic acid, K_1	(10%)
log K_4' -	aliphatic tricarboxylic acid, K_3	(12%)
	aliphatic dicarboxylic acid, K_2	(10%)
	dialkyl malonic acid, K_2	(6%)
	amino acid, K_1 } o-hydroxybenzoic acid, K_2	(32%)

This combination of acids forms the basis of model (i) in Figure 9.4, the amino acid/o-hydroxybenzoic acid (32%) being split as 10% aspartic acid and 22% salicylic acid. Model (ii) in Figure 9.4 is a variation of this combination of

simple acids. The dicarboxylic acid (K_1) has been replaced by a monoprotic acid, and the proportion of salicylic acid increased to adjust for the loss of dicarboxylic acid (K_2) in this region.

A third type of combination of simple carboxylic acids may represent the situation where the value of log K_1' is $\underline{c}$ 6. The model ascribes the weakest acidic groups to tricarboxylic acids (K_1). For example,

log K_1' - aliphatic tricarboxylic acid, K_1

log K_2' - aliphatic dicarboxylic acid, K_1

log K_3' - aliphatic tricarboxylic acid, K_2
aliphatic monoprotic acid, K_1

log K_4' - aliphatic tricarboxylic acid, K_3
aliphatic dicarboxylic acid, K_2
amino acid
o-hydroxybenzoic acid, K_2

Examples of this combination of simple carboxylic acids are models (III), (iv) and (v) in Figure 9.4. SEE ERRATA

Thus, Cu(II) complexing by fulvic acid can be described by mixtures of simple carboxylic acids. Further, the proportions of acidic groups in these mixtures of acids are in similar proportions to those postulated for fulvic acid.

9.3 FLUORESCENCE QUENCHING STUDIES

When an electron returns from the lowest vibrational level of the first excited singlet state to the vibrational levels of the ground state, emitting a photon with energy equal to the difference between the two energy levels, fluorescence emission is observed.

Any process that results in a decrease in the fluorescence efficiency of a molecule is termed fluorescence quenching (FQ). These processes divert the energy into channels other than fluorescence (e.g. intersystem crossing: transition from the lowest vibrational level of a singlet state to an upper vibrational level of a triplet state). Processes which cause changes in the ground state concentration of the fluorescent species (e.g. complexing, dissociation) are not true quenching processes (Hercules, 1966), but do result in a loss of fluorescent emission.

Fulvic acids are among the fluorescent materials found in natural waters. This fluorescence can be quenched by complexing to paramagnetic metal ions which promote intersystem crossing. Thus, FQ experiments offer a technique to monitor metal-ligand complexing.

Fluorescence experiments have several advantages over other techniques used to measure metal-ligand complexing. Firstly, unlike ASV and ISE techniques, FQ measures the concentration of free (fluorescing) ligand. Secondly, the technique is very sensitive, capable of detecting less than 1 mg/ℓ fulvic acid (Saar & Weber, 1982). The major disadvantage of the technique is that it is only applicable to paramagnetic metal ions.

In this work, the complexing of copper(II) by fulvic acids was studied using FQ. The experiments were performed

in conjunction with the ISE studies (Section 9.2), and involved monitoring the fluorescence intensity of a Cu(II) - fulvic acid solution as a function of pH. A direct measure of the uncomplexed Cu(II) was obtained from the ISE measurements, and was compared with the uncomplexed (fluorescent) ligand measured by FQ.

9.3.1 *Results*

The effect of pH on the fluorescence intensity of fulvic acid is shown in Figure 9.6. The intensity of fluorescence as a function of pH (pH 2.7 - 6.5) was measured relative to the intensity at pH 6.5. The fluorescence intensity increased with increasing pH, and reached a limiting value above pH 5. The greatest change occurred between pH 2.7 and 3.5.

The FQ of fulvic acid (1.8×10^{-4} M COOH) by Cu(II) was measured for two different concentrations of Cu(II), 4.0×10^{-5} M and 9.0×10^{-6} M. Four fulvic acid samples were studied (FA1, FA2, FA4, FAI). The results for these samples are shown in Figure 9.7. The fluorescence of Cu(II) - fulvic acid solutions was measured relative to ligand-only at the same pH.

For the 1:4.5 (Cu:COOH) solutions, FQ commenced at pH $\underline{c}$ 3. At pH 6.5 the fluorescence intensity was approximately 50% of that in the absence of metal. The quenching appeared to be levelling off above pH 6.5 to a value $\underline{c}$ 40% that in the absence of metal.

For the 1:20 solutions, FQ was much less than for the higher metal concentration. It appeared to be reaching a limiting value at high pH $\underline{c}$ 70% that of the intensity in the absence of metal. FQ commenced between pH 3 and 4.

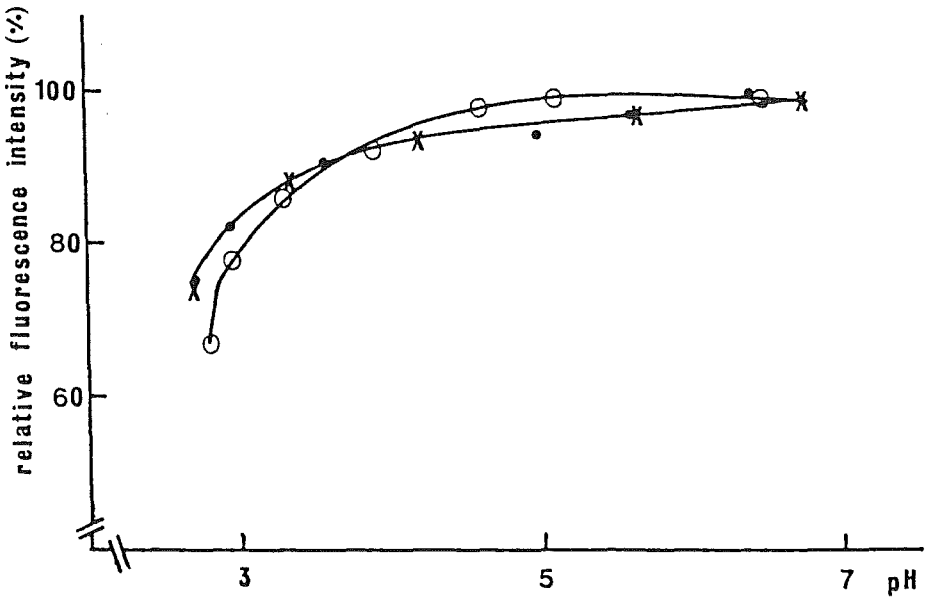


Figure 9.6 Effect of pH on fulvic acid fluorescence. Intensity relative to pH 6.5. FA1, O ; FA2, x ; FA4, ●.

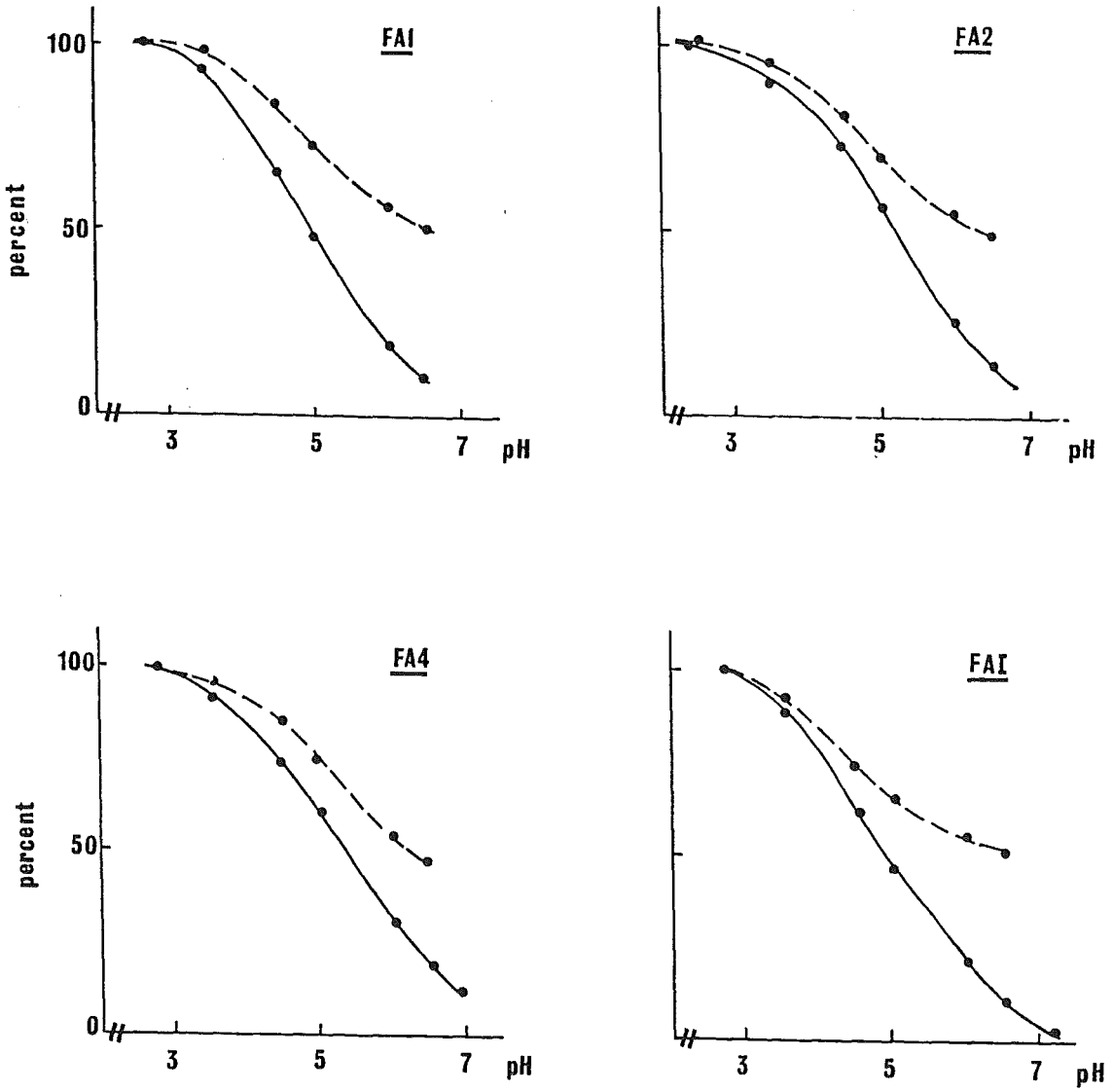
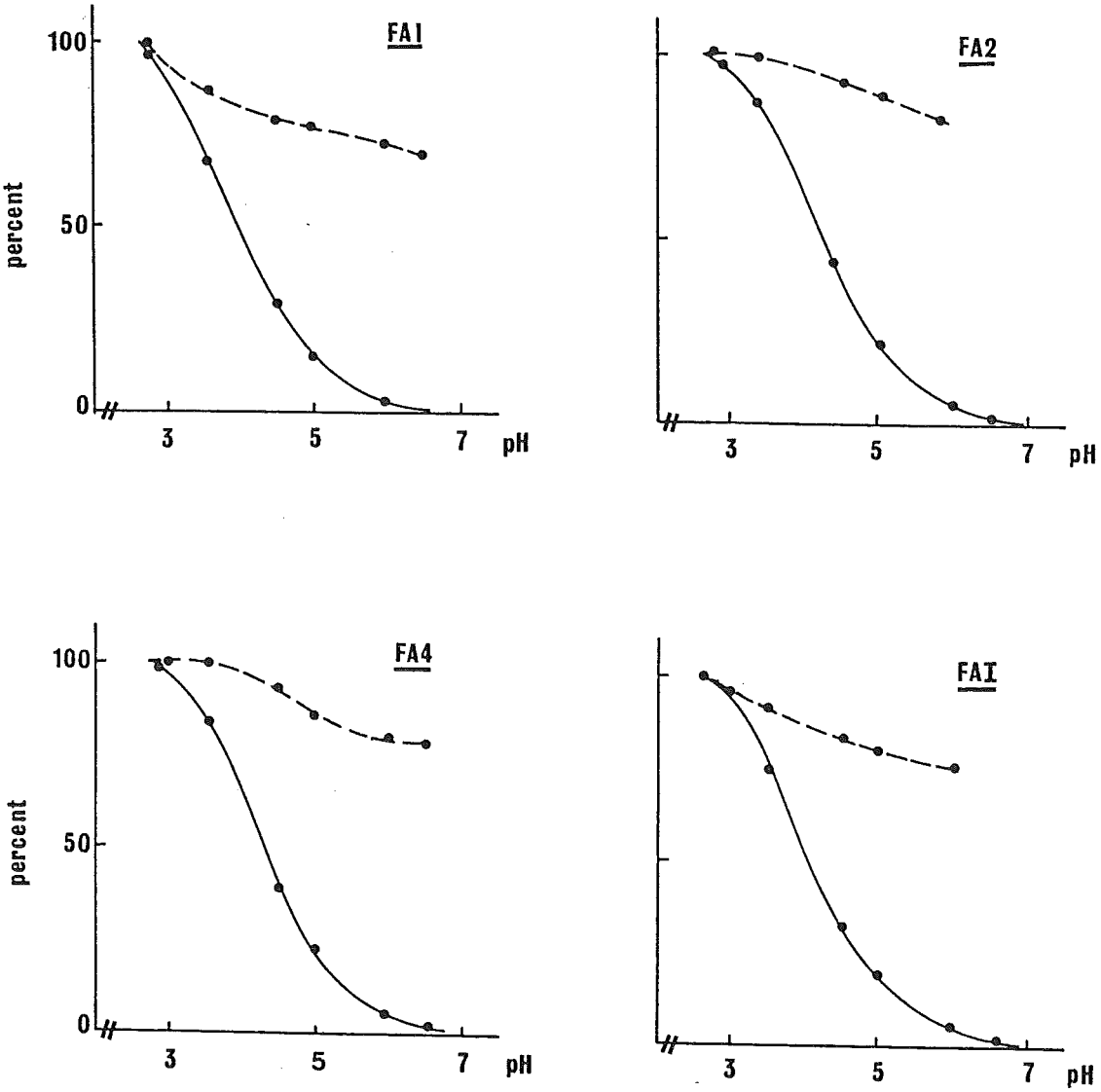


Figure 9.7 Copper(II) binding curves for fulvic acids (1.8×10^{-4} M COOH) determined by ion selective electrode potentiometry, ——— (y-axis, percent free copper) or determined by fluorescence spectroscopy, --- (y-axis, fluorescence intensity relative to no metal).

Figure 9.7(a) Cu(II):COOH 1:4.5.

Figure 9.7(b) Cu(II):COOH 1:20.



9.3.2 *Discussion*

(a) Ligand Fluorescence

Ghosh and Schnitzer (1980) reported fluorescence excitation spectra for fulvic acids. They observed excitation peaks at 360 nm and 465 nm (emission wavelength 520 nm). Underwood et al. (1981) found two emission maxima for fulvic acid; one at 480 nm with an excitation maximum at 370 nm, and another at 520 nm with an excitation maximum at 465 nm. Saar and Weber (1980) reported fulvic acid fluorescence spectra with broad, featureless emission at 445 - 450 nm after excitation at 350 nm.

In this work, fulvic acid solutions and Cu(II) - fulvic acid solutions were excited with 360 nm radiation, and the emission was monitored at 460 nm.

The pH dependence of fulvic acid fluorescence intensity (Figure 9.6) has been noted by several workers (Ghosh & Schnitzer, 1980; Underwood et al., 1981; Laane 1982; Visser, 1983; Saar & Weber, 1980; Cabaniss & Shuman, 1986).

Ghosh & Schnitzer (1980) studied the effect of pH on fluorescence intensity for 0.01% wt/volume solutions of fulvic acid. Excitation maxima at 360 nm and 465 nm (520 nm emission) showed fluorescence intensity increasing with rising pH. For the 360 nm excitation maxima solutions at pH 6.50, 3.50 and 2.00 had 98%, 85% and 50% of the intensity at pH 9.50. Thus, these authors also observed the greatest change in fluorescence intensity occurring at low pH. They ascribed this to an increase in ligand protonation, to increasing particle association (hydrogen bonding), and to coiling of the molecule as the pH is lowered.

Saar and Weber (1980) observed that the emission intensity of fulvic acid (at 445 - 450 nm) was dependent on pH. The intensity increased rapidly from pH 2 to 5, then decreased slightly to pH 8.

Cabaniss and Shuman (1986) also observed a rise in fluorescence intensity up to pH $\underline{c}$ 5, then a slight decrease at higher pH, whereas Laane (1982) observed the intensity increasing with pH but no maximum.

The effect of pH on fulvic acid fluorescence may be ascribed to ligand protonation/deprotonation reactions. The emission wavelength of the protonated ligand need not be the same as for the deprotonated ligand. For example, 2-naphthol fluoresces at 359 nm, whereas the 2-naphtholate anion fluoresces at 429 nm (Seitz, 1981). If the emission is monitored at a fixed wavelength (say, 429 nm) then only the anion will contribute to the observed fluorescence intensity. When the ligand is protonated (as the pH is lowered) the concentration of anion will decrease, and a loss of intensity at 429 nm will result.

For fulvic acid, the sharp decrease in fluorescence is observed at low pH (< 3.5). Carboxyl groups of subsituted phenolics (e.g. salicylic acid) would protonate below pH 3.5, lowering the concentration of the anion and hence may decrease fluorescence intensity. The decrease in fluorescence could not be attributed to phenolic group protonation because phenolic groups would already be completely protonated at this pH. SEE ERRATA

The decrease in fluorescence above pH 5 observed by Saar and Weber (1980) and Cabaniss and Shuman (1986) may be the result of deprotonation reactions of the excited state.

Changes in acidity upon excitation have been observed for a wide variety of ionizable organic compounds (Hercules, 1966). In general, phenols become stronger acids by 5-6 pK units in the excited state (Seitz, 1981). Proton transfer reactions are extremely rapid and can occur to a significant extent during the lifetime of an excited state. Consider the fluorescent phenol, ROH, emitting radiation at $h\nu'$. The ground state can be excited to its lowest excited singlet state ROH*,

$$ROH + h\nu \rightarrow ROH^*$$

This excited state can either (i) emit fluorescent radiation after decay to the lowest vibrational level of the first excited singlet state,

$$ROH^* \rightarrow ROH + h\nu'$$

or (ii) ionize to produce an excited state ion,

$$ROH^* \rightarrow RO^{-*} + H^+$$

The excited state ion can then emit its own characteristic fluorescent radiation, $h\nu''$

$$RO^{-*} \rightarrow RO^- + h\nu''$$

The net outcome of excited state ionization of a phenol is that the intensity of molecular fluorescent radiation ($h\nu'$) is diminished at a pH lower than would be expected for ground-state ionization ($ROH \rightarrow RO^- + h\nu \rightarrow RO^{-*} \rightarrow RO^- + h\nu''$).

(b) Metal-Ligand Complexing

The effect of Cu(II) complexing with fulvic acid is to decrease the fluorescence intensity. A similar effect occurs when H^+ complexes (i.e. when the ligand is protonated). As the pH is raised and the Cu(II) begins to bind to fulvic acid, the fluorescence intensity will (i) decrease due to quenching by a paramagnetic metal ion, and (ii) increase due to ligand deprotonation. Thus, to observe solely the effects of Cu(II) binding, the fluorescence intensity must be measured relative to ligand at the same pH.

The Cu(II) - fulvic acid binding curves are shown in Figure 9.7 for (a) 1:4.5 and (b) 1:20 ratios of Cu(II):COOH. The binding curves for both ISE and FQ studies are shown for comparison. For the FQ studies, the y-axis is % fluorescence intensity (relative to ligand-only). For the ISE studies, the y-axis is % uncomplexed Cu(II).

Consider firstly the 1:4.5 Cu(II):COOH titration (Figure 9.7a). FQ begins at pH $\underline{c}$ 3, and is coincident with the commencement of Cu(II) complexing, as indicated by the ISE binding curves. This implies that fluorescent functional groups are influenced by Cu(II) binding from the commencement of complexing.

Fluorescence in humic substances is likely to be due to either aromatic systems substituted by electron-donating groups, or to highly conjugated aliphatic systems. The general rule for effects of substituents on aromatics is that meta-directing (electron-withdrawing) groups reduce fluorescence, and ortho-para-directing (electron-donating) groups enhance fluorescence (Seitz, 1981). For example, the electron-donating groups -OH, $-OCH_3$, $-NH_2$, -NHR and

$-NR_2$ cause an increase in fluorescence intensity. Electron-withdrawing groups such as $-\overset{\overset{O}{\|}}{C}OH$, $-\overset{\overset{O}{\|}}{C}H-$, $-\overset{\overset{O}{\|}}{C}R$, $-\overset{\overset{O}{\|}}{C}OR$, and $-NO_2$ cause a reduction in fluorescence intensity. Thus the aromatic fluorescent centres in fulvic acids are likely to be substituted aromatics such as phenolics (e.g. phenol, salicylic acid) and aromatic amines.

From ISE studies (Section 9.2), it was shown that functional groups such as salicylates are unlikely to be involved in Cu(II) binding at low pH. The ISE studies also indicate that amino acids may provide important binding sites for Cu(II) at low pH; many aromatic amino acids are known to fluoresce (e.g. tyrosine, phenylalanine).

FQ in the 1:20 Cu(II):COOH titration (Figure 9.7b) began at low pH; for FA1 and FAI the onset of FQ was coincident with the commencement of Cu(II) complexing. However, in contrast to the 1:4.5 titrations, the fluorescence was quenched to a lesser extend; to $\underline{c}$ 70% of the intensity in the absence of metal. This result suggests that fewer fluorescing binding sites are involved in complexing at this ratio. The result is consistent with the interpretation that at lower Cu(II) loadings the strongest binding sites will be occupied. SEE ERRATA

The change in fluorescence intensity due to complexing at high pH is predicted to be small because essentially all of the metal is complexed by pH 6.5. The fluorescence intensity is observed to reach a limiting value, which may be ascribed to non-coordinated fluorescent centres. Computer modelling of Cu(II) - fulvic acid complexing (Section 9.2) indicated that possible fluorescing moieties (e.g. salicylates)

would not be involved in complexing. The fluorescence need not be 100% quenched because there is an excess of ligand in these solutions.

(c) Non-Bonding Interactions

Ligand fluorescence may be quenched if a metal ion (especially a paramagnetic metal ion) is in close proximity to the fluorescent centre. The metal ion need not be bonded to the fluorescent site, but if present within one or two carbons of the site it may quench the fluorescence (Seitz, 1986).

One non-bonding process which may lead to a decrease in fluorescence intensity is collisional quenching. Collisional quenching is a bimolecular process, dependent on the contact between the excited molecule and the quencher. The fluorescent molecule can be excited to its lowest excited singlet state by absorption of a photon,

$$R + h\nu \rightarrow R^*$$

R* can either (i) emit fluorescent radiation and return to the ground-state,

$$R^* \rightarrow R + h\nu'$$

or (ii) collide with a quencher molecule, thereby losing excitation energy and returning to the ground-state without emission of fluorescence,

$$R^* + Q \rightarrow R + Q$$

Collisional energy may be dissipated by enhancement of intersystem crossing or electron transfer (Hercules, 1966). Either way, the intensity of $h\nu'$ is diminished.

Metal ions, especially paramagnetic ions, are common quenchers of fluorescence even when they do not form complexes with the ground state (Seitz, 1981). Thus, free Cu(II) could be partly responsible for the observed quenching at pH 3. As the pH is increased, the concentration of free Cu(II) would decrease, and the contribution to quenching from collisional processes would diminish. The concentration of free Cu(II) diminishes rapidly between pH 4 and 6.5 (from ISE studies). Hence, by pH 6.5 collisional quenching would be minimal.

It is necessary for the quencher to be in sufficient concentration so that there is a reasonable chance of collision with the excited-state of the fluorescent species. A Cu(II) concentration of 4.0×10^{-5} M (used in 1:4.5 studies) is unlikely to cause collisional quenching. To verify this, increments of Cu(II) were added to an acidified (pH 2.5) fulvic acid solution (1.8×10^{-4} M COOH). The pH was low enough to prevent complexing, so any quenching would be due to free metal ion (collisional quenching). There was no measureable quenching for Cu(II) concentrations 2.5×10^{-6} M to 6.25×10^{-5} M. Saar and Weber (1980) observed no significant collisional quenching of fulvic acid fluorescence at high ($\underline{c}$ 0.01 M) Cu(II) concentrations.

Thus, collisional quenching by free Cu(II) is not responsible for quenching of fulvic acid fluorescence at low pH. The FQ is probably due to Cu(II) bound to sites such as aromatic amino acids.

9.4 POLAROGRAPHIC STUDIES

One major limitation of ISE potentiometry in studying metal-ligand complexing is its lack of sensitivity. In general, metal concentrations greater than 10^{-7} M have to be used, and thus studies of metal speciation in systems such as natural waters is limited.

The technique of polarography, more specifically anodic stripping voltammetry (ASV), offers an advantage over ISE potentiometry because it can measure very low concentrations (10^{-9} M) of metal ions (Florence, 1982). This arises partly because of a "built-in" preconcentration step. Metal ions are reduced at the electrode surface for a fixed length of time (plating step). For more dilute solutions, the plating time can be lengthened. The current measured when the reduced form of the metal is oxidized from the drop is related to the metal concentration.

A further advantage of this teqhnique is that it can measure a wider variety of metal ions than ISE potentiometry; these ions may be studied simultaneously with the appropriate choice of plating potential.

As a consequence of the low levels of metal ions in solution, particular care must be taken to minimize contamination. In this work, all polarographic measurements were carried out in a Class-100 clean room. Solutions were prepared with Milli-Q deionized water, and where the use of concentrated acids was required, ARISTAR chemicals were used.

Four major problems arise when interpreting ASV results; (i) the effect of ligand adsorption on the mercury drop, (ii) direct reduction of complexed metal, (iii)

stabilization of intermediate oxidation states, e.g. Cu^{+}, and (iv) incomplete dissociation of complexes within the time scale of the ASV experiment. Failure to take account of these can lead to erroneous concentrations of metal ions. These four topics will be discussed in Sections 9.4.1, 9.4.2, 9.4.3 and 9.4.4 respectively.

9.4.1 *Fulvic Acid Adsorption*

Organic matter may adsorb to the mercury drop and may have several effects on the resulting polarogram. Ritchie et al. (1981) found that humic acids adsorbed on the mercury drop in the potential range 0.0 to -1.0 V (vs. Ag/AgCℓ/KCℓ(sat.) electrode).

Tensammetric waves occur at the adsorption and desorption potentials of a surfactant; fulvic acid is a surfactant. The waves can be mistaken as polarographic waves of reducible substances. Fortunately, they can be clearly distinguished from reducible metal peaks because they contain no faradaic component. Thus, they will be absent in dc polarographic scans.

An adsorbed layer of organic matter on the surface of a mercury drop may set up a physical barrier around the drop such that metal ion diffusion to the drop is hindered. This will diminish the number of metal ions reduced at the drop surface, and consequently will diminish the observed stripping current. A non-linear relationship between the stripping current and the plating (deposition) time will result.

A completely opposite effect to that caused by a physical barrier may occur. The high concentration of

organic matter on the surface of the electrode could enhance the deposition or stripping currents. This would arise if the adsorbed organic matter concentrated the metal ions in close proximity to the drop surface, as a result of complexing or electrostatic interactions (negatively charged functional groups attracting positively charged metal ions). Enhancement of diffusion currents for metal cations in the presence of adsorbed anionic surfactants has been reported (Anson et al., 1976; Jacobsen & Lindseth, 1976).

Shifts in half-wave potentials ($E_{1/2}$) for a metal ion in the presence of a complexing agent can be used to determine stability constants (Bond, 1980), provided the shift is solely due to complexing. However, shifts in $E_{1/2}$ may also arise when organic matter adsorbs on the electrode surface (Greter et al., 1979; Buffle & Greter, 1979).

The occurence of fulvic acid adsorption on the mercury drop was established in this study.

(a) Tensammetric Waves

Batley and Florence (1976) observed the appearance of a broad wave during a differential pulse ASV study of seawater. The broad wave was found to be pH dependent. The wave was not observed after the seawater samples were irradiated with UV light. Seawater samples contain dissolved organic matter, and adsorption of the organic matter on the electrode surface caused an adsorption (tensammetric) wave.

Differential pulse and differential pulse ASV polarograms were recorded for fulvic acid solutions (2 x 10^{-5} M COOH) at pH 4.8. No tensammetric waves were observed in the potential range +0.25 V to -1.5 V. As this was the maximum potential range that subsequent polarographic scans were to be recorded, it was concluded that tensammetric waves were not to be a problem.

(b) The Effect of Drop Time on Current

If adsorption of fulvic acid on the mercury drop is occurring, then any technique which can vary the contact time of the fulvic acid with the drop would be expected to show some effect of adsorption.

For the differential pulse technique, contact time with the drop can be varied by varying the drop time; the longer the drop time, the longer the contact time. In the absence of adsorption the current ratio, i (fulvic acid present): i (fulvic acid absent), should remain constant, regardless of the drop time. Buffle and Greter (1979) observed a non-linear current ratio with increasing drop time for Pb(II)-humic acid solutions at pH 6.0.

Differential pulse polarograms of a solution containing 5.3 x 10^{-6} M Cu/Pb/Cd and 1.6 x 10^{-5} M fulvic acid COOH at pH 4.8 were recorded for drop times 0.5, 1.0 and 2.0 seconds. The peak height (proportional to current) was measured for each metal and compared with that for a fulvic acid-free solution. In contrast to the result of Buffle and Greter (1979), the current ratio was constant with drop time. This indicated that adsorption was not a problem for contact times less than 2 seconds.

It was noted that Buffle and Greter (1979) used humic acid solutions c 50 times more concentrated than in this study when they observed a non-linear relationship between the drop time and the current ratio. The lower concentration of fulvic acid used in this work was chosen to allow studies at environmentally significant levels.

(c) Effect of Plating Time on Current

In ASV, contact time between the mercury drop and the fulvic acid can be varied by varying the plating time. In the absence of adsorption, one would expect to observe a linear relationship between the plating time and the current, Adsorption may affect the linearity of this relationship.

The surfactant Triton-X100 is known to adsorb on the mercury drop (Jacobsen & Lindseth, 1976). The effect of Triton-X100 adsorption on Cu and Pb ASV waves was studied. A solution containing 6×10^{-8} M Cu/Pb at pH 4.8 was plated for 60, 120 and 240 seconds. The observed stripping currents were compared with those for a solution containing 0.001% Triton-X100. A non-linear relationship between plating time and stripping current was observed in the presence of adsorbed surfactant (Figure 9.8).

The effect of plating time on the stripping current in the presence of fulvic acid has been studied. The solutions were 6×10^{-8} M in metal and 8.5×10^{-6} M in fulvic acid COOH. Plating, at -0.9 V, was varied from 60 to 240 seconds. The stripping current showed a non-linear relationship with plating-time (Figure 9.8). This non-linearity can be interpreted as indicating adsorption of fulvic acid on the mercury drop, affecting the electrode

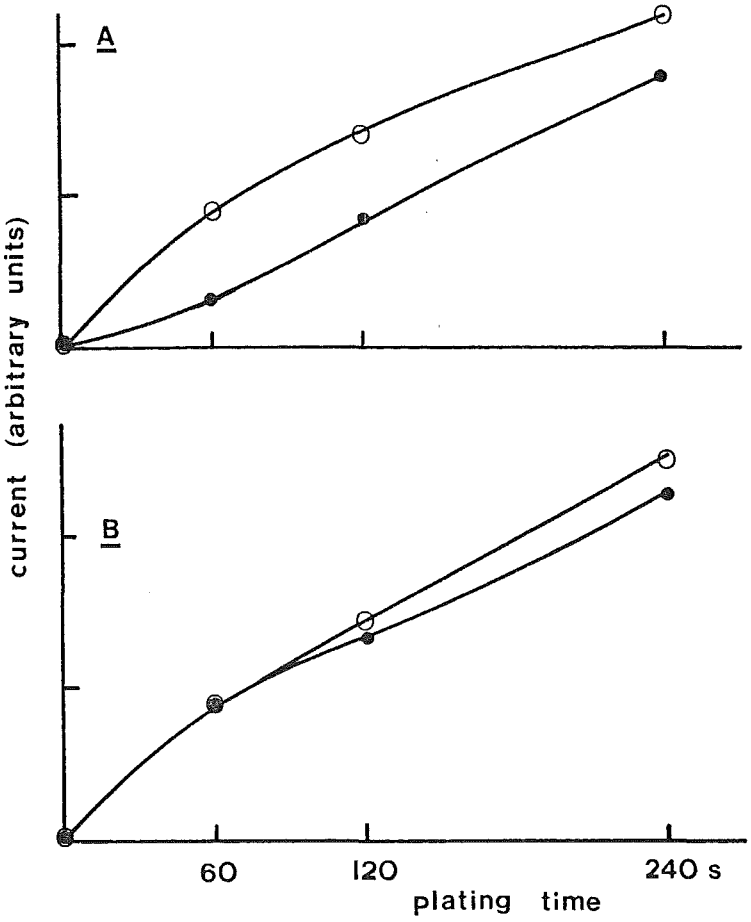


Figure 9.8 Effect of plating time on stripping current for (A) Triton X-100 and (B) fulvic acid (FA2); •, Cu(II) ; O, Pb(II).

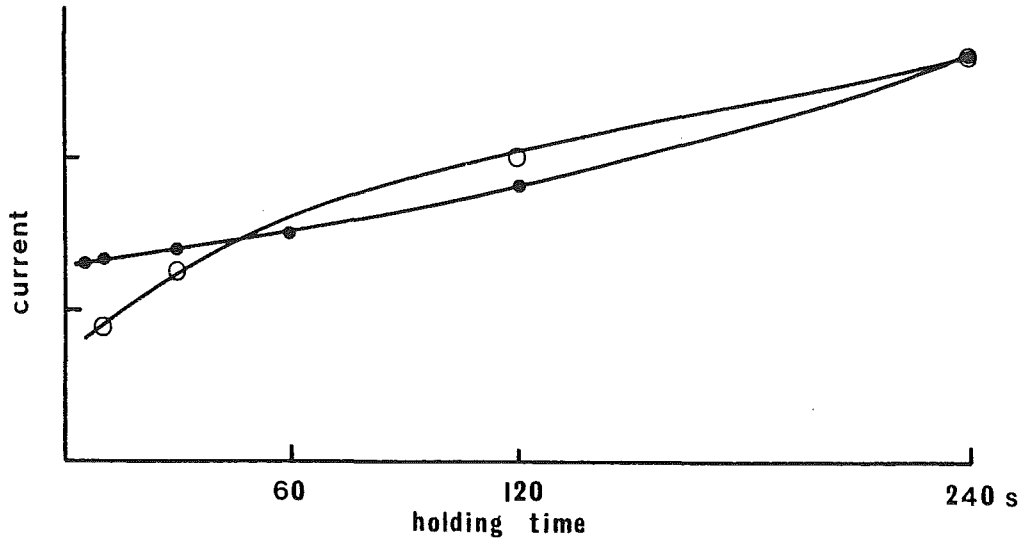


Figure 9.9 Effect on cathodic current of time elapsed at the initial potential before scanning in cyclic voltammetry for Cd(II) in the presence of fulvic acid; •, FA2; O, FAS.

process. The longer the plating time, the more fulvic acid that can be adsorbed.

(d) Cyclic Voltammetry

Contact time with the electrode in cyclic voltammetry can be increased by increasing the elapsed time at the initial potential before the cathodic scan. The initial potential is chosen so that no metal is reduced during the hold period. If adsorption does occur, then the cathodic current should be dependent on this holding time.

Buffle et al. (1976) observed an increase in cathodic peak current with increasing adsorption time (time held at the initial potential) for cyclic voltammograms of Pb - humic acid solutions.

Fulvic acid - Cd solutions (8.2×10^{-6}M COOH, 1×10^{-5} M Cd(II)) were held at an initial potential (-0.4 V) for periods from 5 to 240 seconds prior to recording the voltammograms. The cyclic voltammograms showed a strong dependence on the hold time; the cathodic current was increased by 40-60%, in the presence of fulvic acid at 240 seconds (Figure 9.9).

CONCLUSIONS

Results from ASV and cyclic voltammetry experiments indicate that adsorption of fulvic acid on the mercury drop does occur and will affect the current observed in polarographic studies of metal-fulvic acid complexing. The effect of adsorption on the observed current will have to be accounted for before making meaningful interpretations of experimental data.

The dropping mercury electrode differential pulse experiments indicate that adsorption is not a problem within the time scale of the differential pulse electrode process. The contact time is much shorter (0.5 - 2.0 seconds) than the plating or holding times of the ASV or cyclic voltammetry experiments (5- 240 seconds).

9.2.4 *Pseudopolarograms*

One condition that must be fulfilled if the observed stripping current is to give a direct measure of the free metal ion concentration, is that the metal-organic complex be non-reducible and non-labile at potentials close to the reduction potential of the uncomplexed metal. The presence of reducible complexes (i.e. where electrons are added directly to the complex, before dissociation of the complex) can be detected from the effect of plating potential on stripping current. Such plots are called pseudopolarograms.

The pseudopolarogram of a solution containing non-reducible complexes will show the classical polarographic shape, i.e. current increasing from zero to a limiting value over a small range of plating potential. At any plating potential, the stripping current will be either the limiting current or a fraction of the limiting current, dependending on the plating potential with respect to the half wave potential of the metal ion.

The pseudopolarogram of a solution containing a reducible complex will show continuously increasing current with increasing plating potential.

To minimize the contribution of reducible complexes to the deposition current for free metal, plating should be

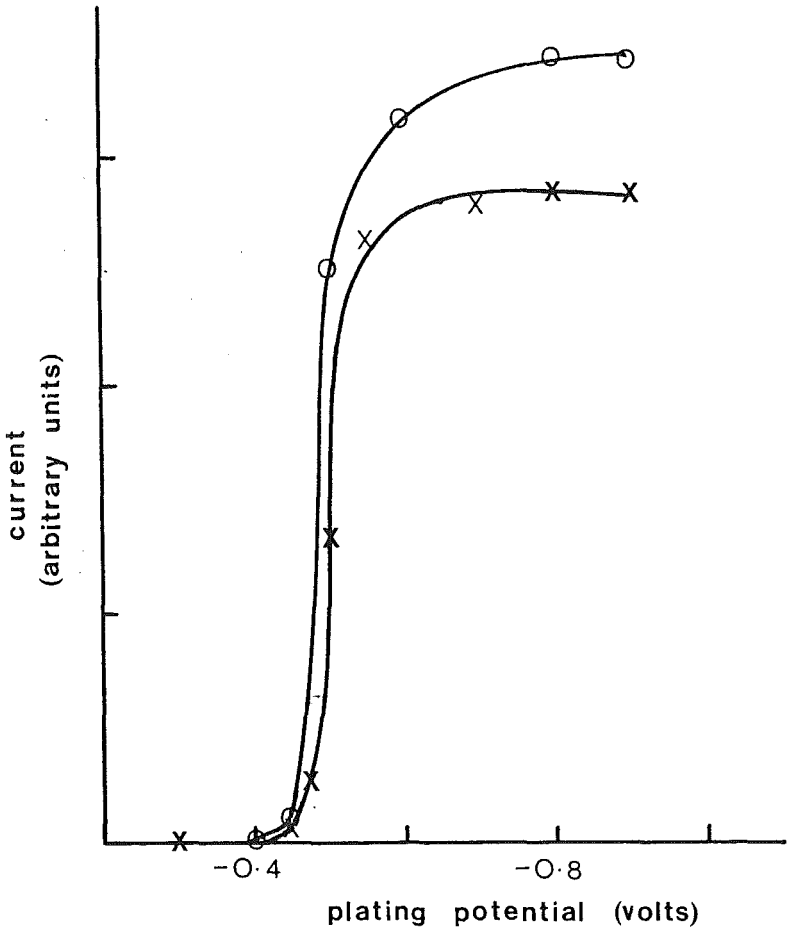


Figure 9.10 Pseudopolarograms for Pb(II), O, and Pb(II)-fulvic acid (FA2), x (2 $x10^{-7}$ M Pb(II), $1.8x10^{-5}$ M COOH, pH 4.8).

carried out at a voltage just sufficiently negative to reduce all of the uncomplexed metal, i.e. the minimum voltage at which the current reaches the limiting current. As a consequence of this, it is not advisable to analyse for several metals simultaneously. For example, if Pb(II) and Cu(II) ions are in solution, then plating at a potential sufficiently negative to reduce all uncomplexed Pb(II) may also be sufficiently negative to directly reduce Cu(II) complexes. Hence the stripping current for Cu(II) would be enhanced.

Pseudopolarograms for Cu(II) and Pb(II), in the presence and absence of fulvic acid, were constructed. The metal solutions (2×10^{-7} M, pH 4.8) were plated at voltages ranging from -0.6 to +0.1 V for Cu(II), and -0.9 to -0.3 V for Pb(II). Metal-ligand solutions (ligand = 1.8×10^{-5} M COOH) were plated over the same voltage range. The pseudopolarograms of metal-only solutions showed the classical polarographic shape, the current rising from zero to a limiting value over a small voltage range. For both Cu(II) and Pb(II) solutions in the presence of fulvic acid, the pseudopolarograms were similar in shape to metal-only curves. The current did not increase continuously with increasing plating potential. The pseudopolarograms for Pb(II) are shown in Figure 9.10.

The curves were constructed for solutions at pH 4.8, a pH at which fulvic acid will form complexes with Pb(II) and Cu(II) (see Sections 9.4.3 and 9.4.4). The difference in limiting current between the metal-only solution and metal-ligand solution ($-E_p > 0.6$ V) can be attributed to the presence of ASV non-labile complexes.

It appears that direct reduction of Cu(II) and Pb(II) - fulvic acid complexes does not occur at voltages close to the reduction potential of the uncomplexed metal. Thus, the stripping current for uncomplexed metal can be determined without interference from direct reduction of complexes.

Anodic Stripping Voltammetry

Anodic stripping voltammetry (ASV) involves depositing (reducing) analyte onto a mercury drop electrode for a predetermined period of time. The reduced species is then stripped (oxidised) from the drop by applying an increasingly positive voltage. The current produced as the species is oxidised is measured as a function of the applied voltage.

The ASV experiment may be divided into two steps, (a) the deposition step, and (b) the stripping step. Processes occurring at each of these steps will determine the observed stripping current.

For example, in a solution containing reducible metal ions and a complexing ligand, the concentration of metal deposited will be dependent on the proportion of complexed and uncomplexed metal. Complexed metal is not reducible unless either the complex can dissociate within the time scale of the ASV experiment, or the complex itself is directly reducible. The deposition process may be further complicated by the effect of adsorbed ligand on the mercury drop surface.

The observed current resulting from oxidation of metals from the mercury drop may be influenced by processes such as stabilization of intermediate oxidation states,

or formation of a variety of metal-ligand complexes with slightly different stabilities.

To gain a better understanding of the processes affecting the deposition (or stripping) step, without the complication of processes occurring in the stripping (or deposition) step, the two steps were considered separately and are discussed in Sections 9.4.3 and 9.4.4.

9.4.3 *The Stripping Process*

The observed stripping current in an ASV experiment is a function of the number of electrons involved in the oxidation process. A decrease in stripping current relative to a metal-only solution may arise if an intermediate oxidation state of the metal can be stabilized during the oxidation process. For example, copper is usually oxidized from the mercury drop to Cu(II), a 2 electron process. However, in the presence of chloride ions, the copper is oxidized to Cu(I), where it is stabilized by chloride, and the resulting stripping current will be lower; this situation arises in the analysis of sea water.

A decrease in stripping current may also indicate that some of the metal has formed non-labile complexes and can no longer be reduced.

To observe the stripping process in the absence of possible complications from the plating process (e.g. adsorption, complex formation, kinetic currents), Cu(II) or Pb(II) were plated onto the mercury drop from a ligand-free solution. This ensured that the same amount of metal was plated onto the drop for each experiment. Ligand was then added to the solution immediately prior to the oxidation

step, and the metal stripped into the ligand solution, at a pH where complexing was possible.

It is possible to distinguish M(I) from M(II) oxidation products from the peak area. The peak area is a measure of charge; $q = \int i \, d(t)$. Therefore the peak area observed when the metal is oxidised to M(I) will be half that for metal oxidised to M(II).

To confirm this peak area dependence on oxidation state, copper was stripped into a solution containing nitrilotriacetic acid (NTA). NTA forms complexes with Cu(II) and does not stabilize the Cu(I) oxidation state. Shifts in $E_{1/2}$ were observed in the presence of NTA, consistent with complex formation. The peak area in the presence of NTA was the same as in the absence of NTA, indicating that the copper was being oxidised to Cu(II). In contrast, when copper was stripped into a solution containing chloride ions, the peak area was halved. This indicated that copper was oxidised to Cu(I)

Stripping into Fulvic Acid Solutions

Copper or lead (plated from 1.2×10^{-7} M solutions) were stripped into solutions of fulvic acid (FA1, FA2, FA4, FAI, FAH, FAS; 1.7×10^{-5} M COOH) at pH 4.8 (complexing) and pH 1.7 (no complexing). At pH 4.8, the copper and lead waves were broad and shifted to more negative voltages, indicative of complexing. However, in each case the peak area for copper stripped into fulvic acid solution was greater than that for metal-only solutions.

Enhanced Cu(II) peaks were also observed when the complexing ligand was citrate.

The possibility of adsorbed fulvic acid enhancing the observed current was considered. Although the fulvic acid was not present during the plating process, it was in contact with the electrode for a significant time during the stripping step. The fulvic acid was added to the polarographic cell during the last 10 seconds of plating. This ensured ample time for mixing without altering significantly the concentration of reducible metal. Following the plating step the stirrer was turned off and the solution allowed 15 seconds to become stationary. The stripping step was then begun, scanning the voltage at 5 mV/s (from -0.9 V). Thus, some 200 seconds elapsed before the potential became sufficiently positive for copper to be oxidised. In Section 9.4.1c it was shown the fulvic acid adsorption does occur over this time span. Thus, adsorbed fulvic acid could concentrate metal in the vicinity of the drop where it could be reduced, and result in an enhanced stripping current. However, decreasing the contact time of fulvic acid with the drop, by increasing the scanning rate, did not significantly change the enhancement effect.

Another possible explanation for the increased current is that fulvic acid enhanced the movement of oxidised metal away from the drop surface. Consider the processes that occur during the stripping step. Firstly, the reduced metal must diffuse from the interior of the mercury drop to the surface where it can be oxidised. An equilibrium between the oxidised and reduced metal is set up on the drop surface. Further metal can be oxidised only when oxidised metal on the surface is removed. In the absence of ligand, this would be by diffusion. In the presence of

ligand at a pH where it can complex, the movement of metal away from the drop may be enhanced. This would allow more metal than usual to be oxidised, and hence the current would be enhanced.

If this explanation is valid, then the same experiment carried out in acidic solution, where complexing was not possible, should result in equal peak areas in the presence and absence of ligand. It did not; peak areas were still enhanced in the presence of ligand. Thus, enhancement of Cu(II) peaks in the presence of fulvic acid is not due to enhanced movement of oxidised metal away from the drop by complexing.

These two previous stripping experiments were performed using different pulse ASV. The differential pulse technique offers the possibility of redeposition of metal back into the mercury drop during the reverse pulse. This would then be stripped again at a later pulse, enhancing the current. Ordinarily, this type of enhancement may not cause any problems. Indeed, it could be used to advantage. However, if there was a mechanism by which the oxidised metal could be concentrated in the vicinity of the drop, then the observed current could be enhanced erroneously.

SEE ERRATA

The presence of adsorbed fulvic acid may provide the mechanism for concentrating "newly" oxidised metal. The mode of concentration cannot be through complexing because the enhancement is observed at low pH (pH 1.7). It could be through some type of electrostatic attraction, or by forming a physical barrier preventing movement of metal away from the mercury surface.

The possibility of redeposition of metal following oxidation can be avoided by using a non-pulse mode of ASV, e.g. sampled dc ASV. The stripping experiment into fulvic acid was repeated, at pH 4.8 and 1.7, using sampled dc ASV. The peak areas in the presence of fulvic acid were found to be the same as those for metal-only solutions, indicating that only M(II) complexes were formed. Further, this result indicates that differential pulse ASV is not a suitable mode of polarography for studying systems where the ligand may adsorb on the electrode surface.

Multiple Complex Formation

At pH 1.7 the stripping peaks were unaffected by the presence of fulvic acid. At pH 4.8 changes in the copper and lead waves indicated that complexes were forming as the metal was oxidised from the drop. The peaks diminished in height but broadened, maintaining the same area (Figure 9.11). The peak heights for copper and lead, relative to ligand-free solution, are given in Table 9.1.

Table 9.1 ASV Peak Heights in the Presence of Fulvic Acid[a]

	Copper(%)	Lead(%)
FA1	86	89
FA2	87	90
FA4	89	93
FAI	77	83
FAH	70	86
FAS	-	91

[a]relative to ligand-free solution, ± 5%.

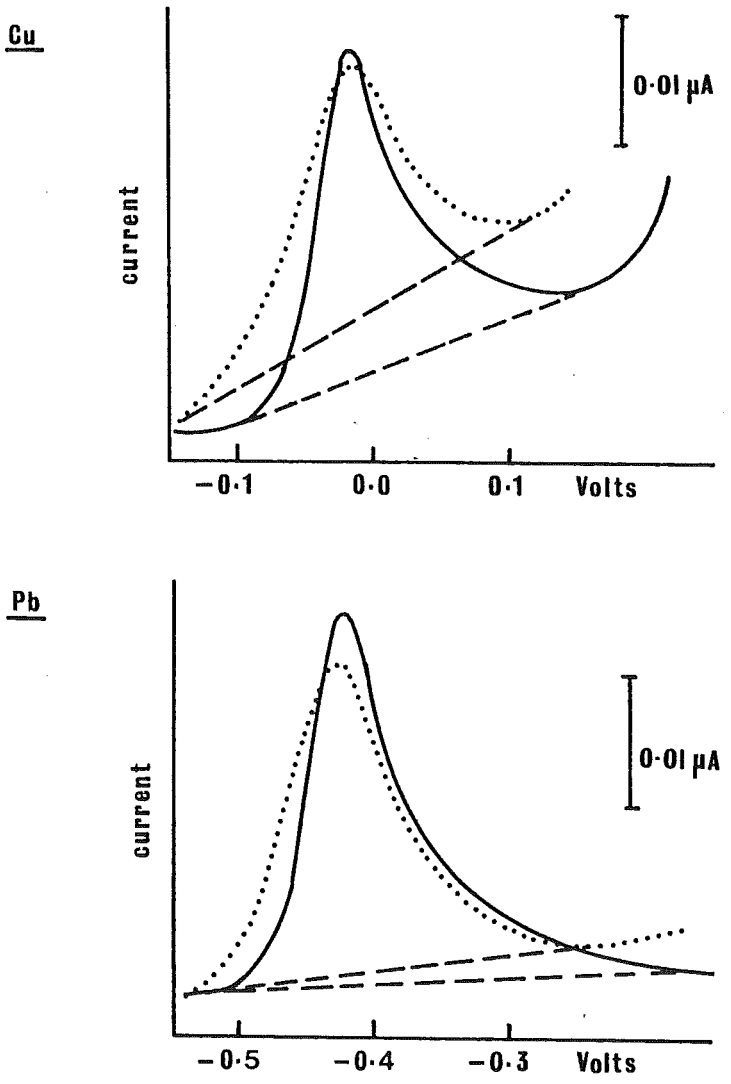


Figure 9.11 Sampled dc-ASV polarograms for 1.2×10^{-7} M copper(II) and lead(II) at pH 4.8. Metal plated from ligand-free solution then stripped into (——) ligand-free solution, or (....) fulvic acid solution (1.7×10^{-5} M COOH).

The greater the decrease in peak height, the broader the peaks must become in order to maintain a constant peak area. The broad nature of the peaks indicates that complexing has occurred; a variety of complexes has formed, each with slightly different stability. If a single complex had formed, then the peak would be the same height as for ligand-free solutions, but would occur at a different potential.

Fulvic acid samples extracted by the acid pyrophosphate/XAD-7 method (FA1, FA2, FA4) showed similar abilities to complex Cu(II) or Pb(II) (Table 9.1). Samples FA4, FAI and FAH can also be compared because they were extracted from the same soil. There is a significant decrease in peak height for both Cu(II) and Pb(II) for the alkali exposed samples (FAI, FAH). The decrease in peak height, or increase in peak width, is indicative of formation of a wider variety of complexes.

9.4.4 *The Deposition Process*

If the rate of dissociation of a complex is very rapid compared with the time scale in which the ASV experiment is performed, then the reduction current (and consequently the observed stripping current) will be almost the same as that for reduction of the aquo ion. Slight differences in currents will arise from slightly different diffusion coefficients of the complex ion and aquo ion. Reduction of metal dissociated from a complex can be distinguished from reduction of the aquo ion by a shift in the reduction potential ($E_{1/2}$). The current resulting from

the reduction of metal ions limited by the rate of diffusion toward the mercury drop is known as a diffusion controlled current.

Another type of metal complex, one that makes no contribution to the reduction current, is the non-labile complex. The rate of dissociation of these complexes is so slow that the equilibrium can be considered frozen during the life time of the Nernstian layer.

A complex of intermediate lability could also exist. for these complexes, partial dissociation can occur during the life time of the Nernstian layer. The current observed for these complexes is not diffusion limited, but limited by the rate of dissociation, and is termed the kinetically controlled current.

SEE ERRATA

The concentration of metal measured by the ASV technique corresponds to the concentration of electro-reducible species. This includes the aquo ion (diffusion controlled) and those species which are sufficiently labile to dissociate in the electrode double layer, i.e. labile complexes (diffusion controlled) and partially labile (kinetically controlled). The proportion of reducible metal in a system may be altered by changing any parameter that changes the residence time of the complex in the electrode double layer, e.g. stirring rate.

The observed stripping current will be a function of processes that occur during the plating step (e.g. reduction of labile complexes, adsorption), and processes that occur during the stripping step (e.g. stabilization of intermediate oxidation states). To compare only those processes occurring during the plating step (i.e. to eliminate variations in the

stripping step process) the stripping step needs to be controlled such that all metal is oxidised to the divalent aquo cation. This can be achieved using medium exchange (Florence, 1986b). Following deposition of metal onto the mercury drop, the sample solution is replaced with a new supporting electrolyte (ligand-free). During this step, the mercury drop is held at the reduction potential. The metal is then stripped into the inert electrolyte; any variations in current reflect only processes occurring in the plating step.

An alternative method to medium exchange is to strip into an acidified solution, i.e. the sample solution is spiked with acid immediately prior to the oxidation step. Thus, labile metal will be plated from the ligand solution but oxidised into a solution where complex formation is prevented. Provided the acid does not stabilize intermediate oxidation states of the metal, the metal will be oxidised to the divalent aquo ion.

It was shown in Section 9.4.3 (Figure 9.11) that following plating from a ligand-free solution, the peaks obtained by stripping into a ligand solution at pH 4.8 were diminished in height but the peak areas were the same as for stripping into an acidified solution, i.e. only divalent ions were formed. Therefore, in this work, when the metal was stripped into a ligand solution at pH 4.8 the stripping current was related to the peak area (not peak height).

To determine the proportion of non-labile Cu(II) and Pb(II) complexes formed with fulvic acid at pH 4.8, solutions containing metal (1.2×10^{-7} M) and fulvic acid

(8×10^{-6} M COOH) were analysed by sampled dc ASV. The solutions were plated for 4 minutes, during which time free metal, labile metal and partially labile metal were reduced. The metal was then oxidised from the drop and the peak area calculated. The solution was then acidified to pH 1.7 ($HCl0_4$), to dissociate any complexes present, and the experiment repeated. The stripping current for the acidified solution represents 100% free metal ion; it includes a term for the effect of fulvic acid adsorption, as does the current observed at pH 4.8. The ratio of peak areas at pH 4.8 and pH 1.7 gives a measure of the proportion of non-labile complexes formed, i.e.

$$\text{fraction of non-labile complexes} = 1 - \frac{\text{peak area at pH 4.8}}{\text{peak area at pH 1.7}}$$

The results for six fulvic acid samples are given in Table 9.2.

Table 9.2 Pb(II) and Cu(II) - Fulvic Acid Complexing

	% non-labile complexes	
	Pb(II)	Cu(II)
FA1	31[a]	24
FA2	21	29
FA4	23	46[a]
FAI	28	43
FAH	30[a]	48
FAS	25	29

[a]mean of 2 experiments, ± 3%.

For this experiment, it was found necessary to acidify the metal solution prior to buffering it to pH 4.8. This was to effect dissociation and/or dissolution of metal-hydroxy polymers that had formed in the stock solution of $CuCl_2$ at pH 4.0. If this was not done it was found that a metal solution plated and stripped at pH 4.8 gave a lower stripping current ($\underline{c}$ 10%) than the same solution that had subsequently been acidified. When the metal ion solution was acidified prior to buffering at pH 4.8, the stripping current at pH 4.8 was the same as that for the acidified solution.

The results in Table 9.2 show a similarity in the percentage of non-labile Pb(II) complexes formed by each fulvic acid (20 - 30% non-labile complexes). The fulvic acid samples that can be directly compared are FA4, FAI and FAH. These samples were all extracted from the same soil, but by different methods. There is a slight, but significant, increase in the proportion of non-labile Pb(II) complexes formed by those fulvic acids exposed to strong alkali conditions (FAI, FAH).

There is a wider range of non-labile Cu(II) complexes formed by the fulvic acids (24 - 48%). The fulvic acids can be divided into two groups, those forming 24 - 29% non-labile complexes, and those forming 43 - 48% non-labile complexes. Further, the samples which form the higher proportion of non-labile complexes are all from the same soil. It is noted (Chapters 3 & 7) that these fulvic acids all contain significantly higher nitrogen levels than the other samples (FA4, FAI, FAH; 2.3%). Ligands with nitrogen donors are known to form ASV non-labile complexes with Cu(II) (Figura & McDuffie, 1980).

Complexing from Mixed Metal Solutions

The effect of different metals competing for binding sites was studied for one of the fulvic acid samples (FA4). A solution was prepared containing 8×10^{-6} M fulvic acid COOH, 1.2×10^{-7} M Cu(II) and 1.2×10^{-7} M Pb(II). The peak areas were determined at pH 4.8 and 1.7, for both Cu(II) and Pb(II), to determine the % non-labile metal. These values are compared with those for individual metal-ligand solutions in Table 9.3.

Table 9.3 Complexing in Mixed Metal Solutions

	Individual Metal Solutions	Mixed Metal Solution
Pb(II)	23%	16%
Cu(II)	46%	38%

For both metals the proportion of non-labile complexes decreased. It is inferred that Cu(II) and Pb(II) are competing for some of the same binding sites. Pb(II) is occupying some sites that Cu(II) would if it were the sole metal ion in solution, and vice versa. The percentage decrease in Pb(II) non-labile complexes is greater than the decrease in Cu(II) non-labile complexes. This indicates that Cu(II) forms the stronger complexes, and is least affected by the presence of another metal.

This result clearly shows the misleading interpretation that would arise if the complexing abilities of several metals were determined simultaneously.

Time Dependence of Complex Formation

A time dependent study of Pb(II) and Cu(II) - fulvic acid complexing was performed to determine whether the equilibrium-composition of the solutions changed with time. In the previous plating experiments, solutions were assumed to have reached equilibrium within 15 minutes of mixing (the time taken to deoxygenate the solution). However, it is possible that solution composition may change over a longer period of time. Pb(II) - fulvic acid and Cu(II) - fulvic acid solutions (1.2×10^{-7} M M(II), 8×10^{-6} M COOH) at pH 4.8 were monitored for non-labile complex content over a period of 2 h, the first measurement taken after 2 minutes mixing. No significant change in stripping current was observed for either metal over this time range. Thus, Cu(II) - fulvic acid and Pb(II) - fulvic acid equilibria are established rapidly (within 2 minutes).

9.4.5 *Polarographic Titration of Cu(II) - Fulvic Acid Solutions*

Titration of a metal-ligand solution is a common approach used in studying metal-ligand equilibria. The titration procedure involves either adding metal ions to a ligand solution (or vice versa), or adding OH^- to an acidified metal-ligand solution of fixed total metal and ligand concentrations. The extent of complexing varies during the titration, and can be used to determine stability constants and complexing capacities. The course of the titration can be followed by any method that can distinguish complexed from uncomplexed metal (or ligand).

To compare the ASV technique with a technique that measures only the free metal ion (e.g. ISE), correction must

be made for dissociation of metal complexes during the ASV experiment. Stripping currents can be corrected for the contribution from kinetic currents, but the procedure is involved (Shuman & Cromer, 1979).

The methods of ISE potentiometry and polarography were compared by titrating an acidified Cu(II) (4.0×10^{-5} M) - fulvic acid (1.8×10^{-4} M COOH) solution with alkali. The total concentration of Cu(II) was selected so that adequate sensitivity was achieved for both techniques. The solutions were titrated between pH 4 and 7.5 with alkali, then back titrated with acid to pH 2.4. The titration cell was set up as for ISE titrations (pH electrode pair, ISE pair), and the emf of the ISE monitored as a function of pH. Aliquots of solution were removed from the titration cell over a range of pH values, and analysed for labile metal content by polarography (sampled dc DME and sampled dc ASV). Following polarographic measurements at each pH, the solution was returned to the titration cell. The results are shown in Figure 9.12.

The ISE method measures the concentration of free (aquo) metal ion in solution. The binding curves indicate that complexing commences between pH 3.0 and 3.5, and that less than 10% free metal remains above pH 7.

The polarographic binding curves also indicated that complexing commenced at approximately pH 3.5. However, these curves did not follow closely the ISE binding curves. They indicated that more "free" metal was in solution at a given pH. This arises because the polarographic technique measures labile metal, of which free metal is only a portion. The additional "free" metal measured is due to labile and partially labile complexes.

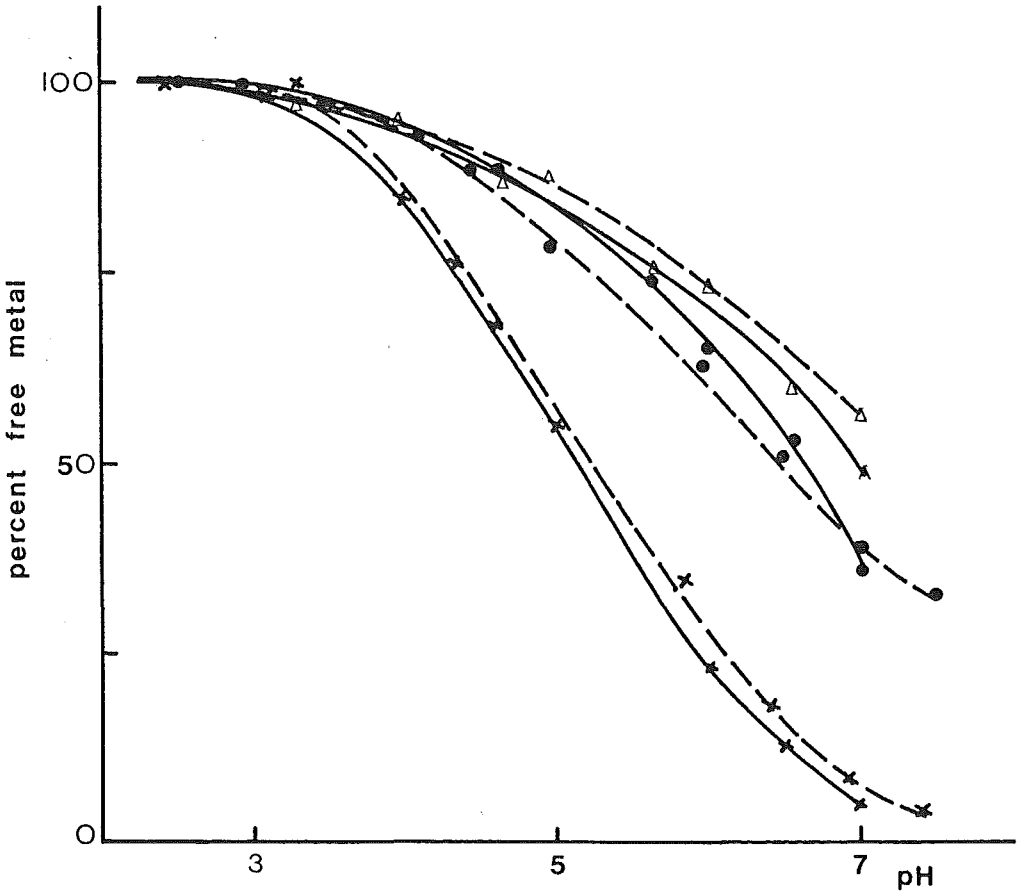


Figure 9.12 Polarographic titration of copper(II)-fulvic acid solution (4.0×10^{-5} M Cu(II), 1.8×10^{-4} M COOH). —— , FA2; --- FA1.

Percent free metal determined by sampled dc-ASV (●), sampled dc-dropping mercury electrode (Δ), ion selective electrode (x).

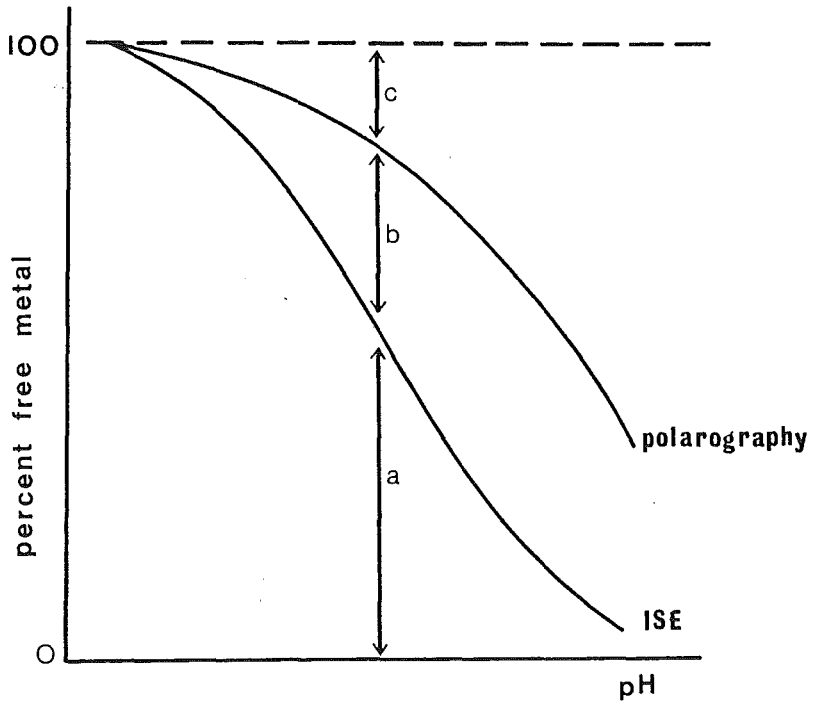


Figure 9.13 Schematic comparison of percent free metal determined by ion selective electrode (ISE), and polarogrpahy.
a, free metal ; a + b, polarographic 'labile' metal ; c, non-labile metal.

Comparison of ISE and polarographic binding curves allows one to estimate the proportion of non-labile complexes, labile complexes and free metal. This is shown diagrammatically in Figure 9.13. At any given pH, the metal measured by the ISE is the free metal fraction (a). The 'labile' metal (a+b) is measured by polarography, where (b) is due to dissociation of complexes. The remaining metal (c), not measured by ISE or polarography, is the metal present in non-labile complexes.

The observed distribution of metal between labile and non-labile complexes is dependent on the mode of polarography used. The proportion of labile metal can be increased by using a mode in which the lifetime of the electrode process is increased. This is clearly demonstrated by the ASV and DME results in Figure 9.12. Sampled dc DME has a much longer lifetime than sampled dc ASV, and thus will include more complexed metal as 'labile' metal.

Provided one technique is adhered to, the fraction of 'labile' metal (for those specific conditions) can be used to compare a variety of samples.

CHAPTER 10

SUMMARY AND CONCLUSIONS

The most common methods used to recover fulvic acids from soils involve extraction with strongly alkaline solutions. There has been much debate about the extent of alteration and degradation of organic matter extracted under alkaline conditions.

A milder extraction procedure, avoiding strongly alkaline and acidic conditions, has been developed in this work. Fulvic acids are released from the soil matrix by a mildly acidic solution of pyrophosphate. The pyrophosphate sequesters ferric ions, preventing possible redox alteration of the organic matter. The fulvic acids are efficiently separated from the pyrophosphate extractant and co-extracted metal ions using macroporous XAD resin. The final product is obtained by freeze drying, following cation exchange and membrane filtration.

By comparing the properties of fulvic acids extracted using the acid pyrophosphate/XAD-7 method with those of fulvic acids extracted using alkali, it was possible to assess the extent of alteration caused by alkali treatment. Because of the heterogeneous nature of fulvic acids and the possible variation in composition arising from the source of the fulvic acids, valid comparisons of extraction methods can only be made if the same soil is used throughout.

In this work three fulvic acid samples, all extracted from the same soil (IHSS reference peat), were compared.

One sample had been extracted with alkali (IHSS reference fulvic acid), and another was extracted using the acid pyrophosphate/XAD-7 method. The third sample used for comparison had been acid pyrophosphate/XAD-7 extracted then subjected to controlled alkali exposure.

The samples were compared on the basis of elemental analyses, spectroscopic characteristics, acid-base properties and metal binding properties.

The elemental analysis of the alkali extracted fulvic acid was similar to the acid pyrophosphate/XAD-7 extracted fulvic acid.

Spectroscopic properties

Spectroscopic techniques (ultraviolet, visible, infrared, nmr) did not reveal any major differences between the three samples. Infrared spectra indicated the presence of hydroxyl groups, carbonyl groups, carboxylic acid units, aliphatic and olefinic chains, and possibly amino acid units. There was very little indication of aromatic groups. The appearance of a sharp band at 2350 cm^{-1} when fulvic acid samples were heated to 120^{o} C was consistent with a decarboxylation reaction, and suggested that malonate units may be present in fulvic acid.

Nuclear magnetic resonance spectra (^{1}H and ^{13}C) indicated considerable aliphatic content, substituted with oxygen and nitrogen containing groups such as carboxyl groups and alcoholic groups. The ^{1}H spectra implied a low aromatic content, or the presence of heavily substituted aromatic groups.

Acid-base properties

In alkaline solution fulvic acids are likely to be altered by chemical reactions such as atmospheric oxidation of phenols, condensation reactions between amino acids and activated carbonyl groups, and hydrolysis reactions of ester linkages. These types of reactions could lead to changes in total acidity. A study of the acid-base properties of the acids may indicate whether such reactions have occurred.

The total titratable carboxyl content of the fulvic acid increased when treated with alkali under controlled (oxygen free) conditions; 139 $gmol^{-1}$ COOH for acid pyrophosphate/XAD-7 extracted fulvic acid, 131 $gmol^{-1}$ COOH for alkali treated (72 h, pH 13) fulvic acid. A less prolonged exposure to alkali ($\underline{c}$ 16 h) during alkali extraction of fulvic acid (IHSS reference fulvic acid) did not produce a significant change in the equivalent weight (137 $gmol^{-1}$ COOH). The decrease in equivalent weight upon controlled exposure to alkali is consistent with an increase in carboxyl group density as would arise from ester hydrolysis reactions.

"Titration" constants were determined for each fulvic acid sample by analysis of acid-base titration data in terms of n independent monoprotic acids. A similar numerical approach has been used before (Paxeus and Wedborg, 1985). The validity of the approach was established by analysing data for (i) citric acid, and (ii) a mixture of malonic and phthalic acids. The calculated "titration" constants for these model systems were in good agreement with those reported in the literature, and the calculated concentrations were in agreement with the known solution stoichiometry.

For fulvic acid, the titration data was best fitted

by four monoprotic acids. The results for six fulvic acids were in the range log K_1' 7.2 - 6.5 ($\underline{c}$ 8% of total carboxyl acidity), log K_2' 5.9 - 5.3 ($\underline{c}$ 11%), log K_3' 5.0 - 4.1 ($\underline{c}$ 20%) and log K_4' 3.2 - 2.4 ($\underline{c}$ 60%). The results indicate relatively few (10 - 20%) polycarboxylate moieties with $n \geqslant 3$.

The effect of alkali treatment on "titration" constants was to increase log K_1' and log K_4'. An increase in log K_1' is consistent with an increase in carboxyl group density, as would arise from ester hydrolysis. However, the increase in log K_4' was not expected; an increase in carboxyl group density would be predicted to decrease log K_4'. An increase in log K_4' may arise from the formation of a condensation product between an amino acid (typically pK $\underline{c}$ 2.4) and an amino acid ester, giving rise to a peptide with pK $\underline{c}$ 3.2.

Metal complexing

Changes in the acid-base properties of fulvic acid upon exposure to alkali may also be reflected in their metal binding properties. However, because of the heterogeneous nature of fulvic acid binding sites, interpretation of metal-ligand equilibria is likely to be difficult. Even for relatively simple ligands, equilibrium models are complicated. For example, in the aluminium(III)-citrate system, six complexes are present ($AlHL^{+}$, AlL, $Al(HL)L^{2-}$, AlL_2^{3-}, $AlL_2H_{-1}^{4-}$ and $Al(LH_{-1})_2^{5-}$). Although satisfied with this model, it differed considerably from previously proposed models, highlighting the difficulty faced when intepreting metal-ligand equilibria for even simple ligands. At any given pH, there will be a variety of complexes present.

For fulvic acid, where several binding moieties are

predicted to exist, the number of complexes present at any given pH is likely to be large. Further, as the pH, ionic strength or the ratio of metal : ligand is changed the dominant binding sites may change. The strongest binding sites will be involved in complexing at low pH and low metal loadings. Consequently, the interpretation of metal-fulvic acid binding in terms of a single conditional stability constant is not satisfactory. From such studies conditional stability constants have been interpreted as indicating the presence of salicylate and phthalate binding moieties. These types of binding sites were not substantiated by results obtained in this study.

In this work a comparative study was made of metal complexing by several fulvic acids. Comparisons of metal complexing between fulvic acids, and with model ligands, were made using ion selective electrode, fluorescence quenching and polarographic techniques. Binding curves for copper(II) and lead(II) (% free metal or ligand vs. pH) were determined at fixed metal : ligand ratios.

Ion selective electrode studies

Ion selective electrode (ISE) studies of copper (II) binding suggested that predominantly polycarboxylate moieties such as citrate and malonate were responsible for binding. As the metal loading was increased, the binding curves more closely resembled those for ligands that form weaker complexes than citrate or malonate, e.g. aspartate. This result reflects a range of binding sites with different affinities for metal ions. The most strongly binding sites are involved in complexing initially, followed by increasingly weaker binding

sites at higher concentrations of metal.

The copper(II)-fulvic acid binding curves were more closely approximated by a mixture of competing ligands, than by one single ligand. The mixtures of ligands that most closely mimicked the fulvic acid binding curves also mimicked the calculated "titration" constants and respective concentrations.

The effect of alkali treatment on copper(II) complexing, as determined by ISE potentiometry, was to displace the binding curves to lower pH. This shift indicates that alkali treated fulvic acid forms more stable complexes with copper(II) than does acid pyrophosphate/XAD-7 extracted fulvic acid. The shift is consistent with an increase in carboxyl groups density, possibly from ester hydrolysis reactions.

Fluorescence quenching studies

The quenching of fluorescence when fulvic acid was complexed to paramagnetic copper(II) implied that substituted aromatic units were involved in complexing (or in proximity to complexing sites). This is in contrast to the ISE results which indicated aliphatic polycarboxylate binding sites. Further, infrared and nmr spectra of fulvic acids did not support aromatic character.

Quenching commenced at the onset of complexing (as indicated by ISE studies), indicating that fluorescent centres are involved in copper(II) binding from the commencement of complexing. Quenching was incomplete at a pH high enough for all metal to be bound. This residual fluorescence intensity may be ascribed to non-coordinated fluorescent centres.

Anodic stripping voltammetry studies

Studying metal complexing by anodic stripping voltammetry (ASV) offers the advantage of high sensitivity relative to many other techniques. Unfortunately it also has its drawbacks.

The ASV experiment involves two steps, the deposition step and the stripping step. At each step complications may arise which will affect the observed stripping current. The effects of ligand adsorption, direct reduction of complexes, stabilization of intermediate oxidation states, and the contribution to the deposition current from dissociation of complexes all contribute to the observed stripping current and hence to the inferred "free metal" concentration.

Adsorption of fulvic acid on the mercury drop was found to occur within the timescale of the ASV experiment, but was not a problem when using the dropping mercury electrode mode.

Pseudopolarograms were constructed for copper(II) and lead(II)-fulvic acid solutions at a pH where complexing was occurring. There was no indication of direct reduction of metal complexes.

The procedure for studying metal-ligand complexing by ASV polarographic techniques is based on the assumption that complexes formed do not dissociate at a rate sufficient to contribute to the metal ion reduced at the electrode. Unfortunately this assumption is not valid for fulvic acid. The observed "free metal" current contains a component due to labile metal (diffusion current) and partially labile metal (kinetic current). The measured current represents ASV-labile metal, not solely uncomplexed metal. This was

indicated by comparison of ASV and ISE binding curves for a copper(II)-fulvic acid titration.

Using sampled dc ASV, the fraction of metal in non-labile lead(II)-fulvic acid complexes was observed to range from 20 to 30% for the fulvic acids studied. Alkali exposed fulvic acids formed a slightly higher proportion (c 25-30%) of non-labile lead(II) complexes. The fraction of metal in non-labile copper(II) complexes ranged from 24 to 48%, but there was no significant difference for the alkali exposed fulvic acids.

The observed stripping current is not only a function of the concentration of metal reduced onto the mercury drop, but is also a function of processes occurring during the oxidation (stripping) step, e.g. stablization of intermediate oxidation states, or formation of several complexes with slightly different stabilities. To observe processes occurring during the stripping step in the absence of possible variations caused by the deposition step, copper(II) and lead lead(II) were plated from a ligand-free solution, then stripped into a solution containing fulvic acid.

It was necessary to use a non-pulse mode of ASV for these experiments. A combination of adsorbed fulvic acid and a pulse ASV mode was thought responsible for enhancement of stripping current in the presence of ligand.

At a pH where complexing was possible, the lead(II) and copper(II) waves were broad and shifted to more negative voltages, indicative of complexing. Using sampled dc ASV, the peak areas in the prsence of fulvic acid were the same as those for metal-only solutions, indicating that intermediate oxidation states of the metals were not stablized by fulvic acid. SEE ERRATA

The broad nature of the peaks indicated that a variety of complexes had formed with slightly different stabilities. The broader the peak, the wider the range of stabilities. The peaks were significantly broader (c 10% for Pb, c 18% for Cu) for alkali exposed fulvic acids. This implies that the fulvic acids have been chemically altered during exposure to alkali, generating a wider variety of acidic complexing sites.

The comparison of spectroscopic characteristics, acid-base properties and metal binding properties of fulvic acid samples extracted or exposed to alkali with those of acid pyrophosphate/XAD-7 extracted fulvic acid has revealed some general features of fulvic acids and some differences arising from methods of extraction. Spectroscopic studies, "titration" constant calculations and copper(II) ISE binding studies imply a predominantly aliphatic carboxylate nature of fulvic acids. Chemical alteration caused by exposure to alkali was indicated by changes in acid-base properties (equivalent weight, "titration" constants), and to a lesser extent by changes in metal binding properties.

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APPENDIX A

Subroutines for Determination of Triprotic Acid Protonation Constants

```
      SUBROUTINE PRELIM(YO,X,NO)
      DIMENSION X(1,100),YO(100)
C
C     TO CALCULATE NBAR-OBS
C
C     INPUT DATA---INITIAL TOTAL ACID THI, INITIAL TOTAL LIGAND TLI,
C     TITRANT KOH MOLARITY ALK, TOTAL VOLUME VOL
      READ(5,42)THI,TLI,ALK,VOL
      DO 11 I=1,NO
C
C     CONVERT PH-MEAS TO PH+
C
      PH=(X(1,I)-0.04137)/1.013
      H=10.**(-PH)
      Q=VOL/(VOL+YO(I))
      TL=TLI*Q
      TH=THI*Q
      ALKA=ALK*YO(I)/(VOL+YO(I))
      HYDR=1.007E-14/(0.625*H)
C
C     STORE NBAR-OBS AS YO
C
   11 YO(I)=(TH-H-ALKA+HYDR)/TL
   42 FORMAT(2E10.3,2F10.3)
      RETURN
      END
      SUBROUTINE CALC(X,P,I,Y)
      DIMENSION X(1,200),P(4)
C
C     TO CALCULATE NBAR-CALC
      PH=(X(1,I)-0.04137)/1.013
C
      H=10.**(-PH)
      ABP=P(1)*H*(1.0+2.0*P(2)*H+3.0*P(2)*P(3)*H**2)
      ABB=1.585+P(1)*H*(1.0+P(2)*H+P(2)*P(3)*H**2)
      Y=ABP/ABB
      RETURN
      END
```

APPENDIX B

Subroutines for Determination of Aluminium(III)-Citrate Stability Constants

```
      SUBROUTINE PRELIM(YO,X,NO,ATL,ATM)
      DIMENSION YO(200),X(1,200),ATL(200),ATM(200),ATH(200)
      REAL L
C
C     INPUT DATA *** TOTAL LIGAND=TL, TOTAL METAL=TM, TOTAL VOLUME(ml)=VOL,
C                    MOLE EXCESS ACID=EA, KOH CONCENTRATION=CKOH,
C
      READ(5,42)CKOH,VOL,TL,TM,EA
      WRITE(6,74)
C
C     INPUT DATA *** FOR BUFFER CORRECTION ***
C                    BUFFERS BEFORE TITRATION  Y=SLOPE1*X+CINT1
C                    BUFFERS AFTER  TITRATION  Y=SLOPE2*X+CINT2
C
      READ(5,43)SLOPE1,SLOPE2,CINT1,CINT2
   43 FORMAT(2F6.3,2F8.4)
C
C     CONVERT pH-obs TO pH-meas
C
      CSLOPE=(SLOPE2-SLOPE1)/NO
      CINT=(CINT1-CINT2)/NO
      DO 10 I=1,NO
      AN=I
      X(1,I)=(X(1,I)+(-CINT1+CINT*AN))/(SLOPE1+CSLOPE*AN)
   10 CONTINUE
C
C     CONVERT pH-MEAS TO pH+
      DO 11 I=1,NO
      PH=(X(1,I)-0.04137)/1.013
      H=10.**(-PH)
      X(1,I)=PH
C
C     DILUTION CORRECTION
      Q=VOL/(VOL+YO(I))
      ATL(I)=TL*Q
      ATM(I)=TM*Q
C
C      CITRIC ACID CUMMULATIVE PROTONATION CONSTANTS
      AB1=10.**(5.70)
      AB2=10.**(10.05)
      AB3=10.**(12.95)
C
C     CALCULATE TOTAL ACID (YOBS)
      ALKK=((YO(I)*CKOH)/1000.)-EA
      ALKA=(ALKK*1000.)/(VOL+YO(I))
      ATH(I)=3.*ATL(I)+1.611E-14/H-ALKA
C
C      CALCULATE NBAR
      L=(ATH(I)-H)/(AB1*H+2.*AB2*H**2+3.*AB3*H**3)
      ANBAR=(ATL(I)-L*(1.+AB1*H+AB2*H**2+AB3*H**3))/ATM(I)
C
C      CALCULATE ZL=NUMBER OF PROTONS RELEASED/LIGAND
      ALL=TL*VOL/1000.
      ZL=ALKK/ALL
C
      WRITE(6,18)YO(I),X(1,I),ATL(I),ATM(I),ATH(I),ANBAR,ZL
   11 YO(I)=ATH(I)
   42 FORMAT(F5.3,F7.2,3E10.3)
```

```
   74 FORMAT( ' Y0(I)    X(1,I)     ATL          ATM          ATH          NB
     1AR         ZL ')
   18 FORMAT(2(F6.3,2X),5(E10.4,2X))
      RETURN
      END
      SUBROUTINE CALC(NO,I,X,P,ATL,ATM,SL,SFM,Y,NBAR,F1,F2,PL,AL)
      DIMENSION X(1,200),ATL(200),ATM(200),SL(200),SFM(200),P(10)
      DIMENSION AL(200,20)
      REAL L,NEWL,OLDL,NBAR
C     CITRIC ACID CUMMULATIVE PROTONATION CONSTANTS
      AB1=10.**(5.70)
      AB2=10.**(10.05)
      AB3=10.**(12.95)
      H=10.**(-X(1,I))
C
      IF(I-1)1,1,2
    1 L=ATL(I)/(1.0+AB3*H**3+AB2*H**2+AB1*H)
      FM=ATM(I)/2.0
      GO TO 3
    2 L=SL(I-1)
      FM=SFM(I-1)
    3 CONTINUE
C
C     ALUMINIUM HYDROLYSIS CONSTANTS - OHMAN'S DATA
      BETA1=10.**(-5.52)
      BETA2=0
      BETA3=0
      BETA4=0
      BETA5=(-103.2)
      BETA6=0
      BETA7=10.**(-13.57)
C
C     USE TRIAL VALUE OF L TO SOLVE FOR FM USING NEWTON RAPHSON
      NY=0
   31 CONTINUE
      NX=0
   27 FN=ALOG10(13.)+BETA5+13.*ALOG10(FM)-32.*ALOG10(H)
      IF(X(1,I)-6.0)54,55,55
   54 FN=0.0
      A=0.0
      GO TO 56
   55 A=10.**(FN)
   56 CONTINUE
      B=3.*BETA7/H**4+3.*P(8)*P(6)**3*P(3)**3*L**3/H**4
      C=2.*BETA3/H**2+2.*P(10)*P(3)**2*L**2
      D=1.+P(1)*L*H**2+P(2)*L*H+P(3)*L+P(4)*P(3)*L**2+P(5)*P(4)*P(3)*L**
     12/H+P(6)*P(3)*L/H+P(7)*P(6)*P(3)*L**2/H**2+BETA1/H+BETA2/H**2+
     2BETA6/H**3+BETA4/H**4+P(9)*P(5)*P(4)*P(3)*L**2/H**2
      FX=A+B*FM**3+C*FM**2+D*FM-ATM(I)
      FXP=13.*A/FM+3.*B*FM**2+2.*C*FM+D
      XM=FM-FX/FXP
      PFM=-ALOG10(FM)
      PXM=-ALOG10(XM)
      G=PFM-PXM
      FM=XM
      IF(ABS(G)-0.001)25,25,26
   26 NX=NX+1
      IF(NX-20)27,25,25
C
C
```

```
C      USE FM TO SOLVE FOR L USING NEWTON RAPHSON
   25  DA=3.*P(8)*P(6)**3*P(3)**3*FM**3/H**4
       DB=2.*P(7)*P(6)*P(3)*FM/H**2+2.*P(5)*P(4)*P(3)*FM/H+2.*P(4)*P(3)*
      1FM+2.*P(9)*P(5)*P(4)*P(3)*FM/H**2+2.*P(10)*P(3)**2*FM**2
       DC=1.+P(1)*FM*H**2+P(2)*FM*H+P(3)*FM+P(6)*P(3)*FM/H+
      1AB3*H**3+AB2*H**2+AB1*H
       OLDL=-ALOG10(L)
       N=0
   30  FX=DA*L**3+DB*L**2+DC*L-ATL(I)
       FXP=3.*DA*L**2+2.*DB*L+DC
       Z=L-FX/FXP
       PZ=-ALOG10(Z)
       PFL=-ALOG10(L)
       G=PZ-PFL
C
       L=Z
       IF(ABS(G)-0.001)20,20,21
   21  N=N+1
       IF(N-40)30,20,20
C
C      TEST REFINEMENT OF L
   20  NEWL=-ALOG10(L)
       DIFF=NEWL-OLDL
       IF(ABS(DIFF)-0.001)7,22,22
   22  NY=NY+1
       IF(NY-20)31,7,7
C
C      SAVE L AND FM AS TRIAL VALUES FOR NEXT TIME CALC IS ENTERED
    7  SL(I)=L
       SFM(I)=FM
C
C      CALCULATE TOTAL ACID (YCALC) FROM FREE METAL (FM), LIGAND (L) AND
C      TRIAL PARAMETERS
       FN=BETA5+13.*ALOG10(FM)-32.*ALOG10(H)
       IF(X(1,I)-6.0)50,51,51
   50  FN=0.0
       FNN=0.0
       GO TO 52
   51  FNN=10.**(FN)
   52  CONTINUE
       BA=1.+AB1*L+2.*AB2*L*H+3.*AB3*L*H**2+P(2)*FM*L
       BB=-P(5)*P(4)*P(3)*FM*L**2-P(6)*P(3)*FM*L-BETA1*FM
       BC=-2.*BETA2*FM-2.*BETA3*FM**2-2.*P(7)*P(6)*P(3)*FM*L**2
      1-2.*P(9)*P(5)*P(4)*P(3)*FM*L**2
       BD=-4.*P(8)*P(6)**3*P(3)**3*FM**3*L**3-4.*BETA4*FM-4.*BETA7*FM**3
       Y=BA*H+2.*P(1)*FM*L*H**2+BB/H+BC/H**2-3.*BETA6*FM/H**3+
      1BD/H**4-32.*FNN
C
C      CALCULATE PERCENT DISTRIBUTION OF ALL METAL SPECIES
C      A1H2L=AL1, A1HL=AL2, A1L=AL3, A1L2=AL4, A1L2(OH)=AL5, A1L(OH)=AL6,
C      A1L2(OH)2=AL7, A13L3(OH)4=AL8, A1OH=AL9, A1(OH)2=AL10, A1(OH)3=AL11,
C      A1(OH)4=AL12, A12(OH)2=AL13, A13(OH)4=AL14, A113(OH)32=AL15, A1=AL16
C
       ZF=100./ATM(I)
       AL(I,1)=P(1)*FM*L*ZF*H**2
       AL(I,2)=P(2)*FM*L*ZF*H
       AL(I,3)=P(3)*FM*L*ZF
       AL(I,4)=P(4)*AL(I,3)*L
       AL(I,5)=P(5)*AL(I,4)/H
       AL(I,6)=P(6)*AL(I,3)/H
```

```
      AL(I,7)=P(7)*AL(I,6)*L/H
      IF(P(7).EQ.0.0) AL(I,7)=P(9)*AL(I,5)/H
      AL(I,8)=3.*P(8)*AL(I,6)**3/H
      AL(I,9)=BETA1*FM*ZF/H
      AL(I,10)=BETA2*FM*ZF/H**2
      AL(I,11)=BETA6*FM*ZF/H**3
      AL(I,12)=BETA4*FM*ZF/H**4
      AL(I,13)=2.*BETA3*FM**2*ZF/H**2
      AL(I,14)=3.*BETA7*FM**3*ZF/H**4
      AL(I,15)=13.*FNN*ZF
      AL(I,16)=FM*ZF
      AL(I,17)=2.*P(10)*P(3)**2*FM**2*L**2*ZF
C
C     OTHER USEFUL FUNCTIONS
C
      NBAR=(ATL(I)-L*(1.+AB1*H+AB2*H**2+AB3*H**3))/ATM(I)
      F1=ATM(I)/FM
      F2=ATL(I)/L
      PL=-ALOG10(L)
C
      RETURN
      END
```

APPENDIX C

Determination of Proton-Ligand pH Distribution

```
C      PROGRAM FOR PERCENT COMPOSITION OF LIGAND-ACID SOLUTIONS
C       B VALUES ARE CUMULATIVE CONSTANTS
       REAL L
C      B VALUES ARE CUMULATIVE CONSTANTS
       READ(5,50) B1, B2, B3, B4
       B1=10.**(B1)
       B2=10.**(B2)
       B3=10.**(B3)
       B4=10.**(B4)
       READ(5,51) N,PHO, TL

       WRITE(6,60)
       WRITE(6,61)
       DO 1 I=1,N
       AN=I
       PH=PHO + 0.20*AN
       H=10.**(-PH)
       D = 1. + B1*H+B2*H**2+B3*H**3+B4*H**4
       L=TL/D
       C=100./TL
       HL=L*H*B1*C
       H2L=L*H**2*B2*C
       H3L=L*H**3*B3*C

       H4L=L*H**4*B4*C
       L=L*C
     1 WRITE(6,62)PH,H4L,H3L,H2L,HL,L
    50 FORMAT(4F10.3)
    51 FORMAT(I2,4X,F4.2,E10.3)
    60 FORMAT('PERCENT COMPOSITION FOR POLYBASIC ACID SOLUTIONS')
    61 FORMAT('   PH    H4L    H3L    H2L    HL      L')

    62 FORMAT(6(F5.2,3X))
        CLOSE(5,STATUS='KEEP')
        CLOSE(6,STATUS='KEEP')
       STOP
       END
```

APPENDIX D

Determination of Copper(II)-Ligand pH Distribution

```
C      ***PROGRAM FOR COPPER - LIGAND MIXTURES ****
C     K IS NO. OF LIGANDS ** N IS NO. OF PH INCREMENTS
      REAL L(10),M,ML(100,10),ML2(100,10),MHL(100,10),MHL2(100,10),
     1MH2L2(100,10),MLOH(100,10),M2L2(100,10)
      INTEGER P
      DIMENSION RL(100),RM(100)
      DIMENSION D(10),AK(10,10),SUM(10),NM(100),C1(10),C2(10)
      DIMENSION B1(10),B2(10),B3(10),TL(10),NJ(100,10),TITLE(18,
     110),AH(100),HYD(100,10),XM(100),C3(10)
      DIMENSION PH(100),VOL(100),PM(100)
      NN=1
      READ(5,50) KODE
   51 CONTINUE
      SIGL=0.0
C       K IS NUMBER OF LIGANDS COMPETING FOR METAL ION ****
      READ(5,70) TMI,K
      DO 10 J=1,K
      READ(5,80) (TITLE(P,J),P=1,18)
      READ(5,71) TLI,BA,BB,BC
        WRITE(6,71)TLI,BA,BB,BC
      B1(J)=10.**(BA)
      B2(J)=10.**(BB)
      IF(ABS(BB).LT.0.1)  B2(J)=0.0
      B3(J)=10.**(BC)
      IF(ABS(BC).LT.0.1)  B3(J)=0.0
        WRITE(6,8)B1(J),B2(J),B3(J)
    8   FORMAT(3E10.3)
      DO 16 I=1,7
      READ(5,72) A
      AK(I,J)=A
      IF(ABS(A).GT.0.1) AK(I,J)=10.**(A)
      WRITE(6,69)AK(I,J)
   16 CONTINUE
   10 CONTINUE
      BETA1=4.57E-8
      BETA2=2.69E-11
      READ(5,75) TVOL,ACID
      I=1
    2 READ(5,73) VOL(I),PH(I),KX
      N=I
      I=I+1
      IF(KX)2,2,3
    3 READ (5,76) ALK
      WRITE(6,74) TLI,TMI,N
C
C      BUFFER CORRECTION FOR ELECTRODE DRIFT
C      BUFFERS BEFORE TITRATION     Y=SLOPE1*X+CINT1
C      BUFFERS AFTER TITRATION      Y=SLOPE2*X+CINT2
C
      READ(5,77)SLOPE1,SLOPE2,CINT1,CINT2
      CSLOPE=(SLOPE2-SLOPE1)/N
      CINT=(CINT1-CINT2)/N
C
      DO 11 I=1,N
      NM(I)=0
C
C      CONVERT pH-obs to pH-meas
      AN=I
```

```
      PH(I)=(PH(I)+(-CINT1+CINT*AN))/(SLOPE1+CSLOPE*AN)
C
C       CONVERT pH-meas to pH+
      PH(I)=(PH(I)-0.037)/1.012
C
      TM=TMI*TVOL/(TVOL+VOL(I))
      H=10.**(-PH(I))
      SIG=0.0
      DO 12 J=1,K
      TL(J)=TLI*TVOL/(TVOL+VOL(I))
      D(J)=1.0+B1(J)*H+B2(J)*H**2+B3(J)*H**3
      ZK=K
      SIGX=SIGL*TM/101.0
      L(J)=(TL(J)-SIGX)/D(J)
      SUM(J)=AK(1,J)*L(J)+AK(2,J)*L(J)**2+AK(3,J)*L(J)*H+AK(4,J)*L(J)**
     12*H+AK(5,J)*L(J)**2*H**2+AK(6,J)*L(J)/H
   12 SIG=SIG+SUM(J)
      M=TM/(1.0+SIG)
      J=1
      M=TM/(1.0+SIG+2.0*M*AK(7,J)*L(J)**2)
   40 DO 13 J=1,K
      NJ(I,J)=0
      XL=L(J)
      A=M*2.*(AK(4,J)*H+AK(5,J)*H**2+AK(2,J)+M*AK(7,J))
      B=D(J)+M*(AK(1,J)+AK(3,J)*H+AK(6,J)/H)
      C=-TL(J)
      NX=0
   30 FX=A*XL**2+B*XL+C
      FXP=2.*A*XL+B
      Z=XL-FX/FXP
      NX=NX+1
      IF(Z) 41,41,43
   43 IF(NX-10) 55,55,56
   55 XL=Z
      GO TO 30
   56 PZ=ALOG10(Z)
      PXL=ALOG10(XL)
      G=PZ-PXL
      NJ(I,J)=NJ(I,J)+1
      IF(ABS(G)-0.001)20,20,21
   21 XL=Z
      IF(NJ(I,J)-3) 30,20,20
   20  L(J)=Z
   13 CONTINUE
      SIG=0.0
      DO 14 J=1,K
      SUM(J)=AK(1,J)*L(J)+AK(2,J)*L(J)**2+AK(3,J)*L(J)*H+AK(4,J)*L(J)**
     12*H+AK(5,J)*L(J)**2*H**2+AK(6,J)*L(J)/H
   14 SIG=SIG+SUM(J)
      SM=M
      SS=0
      DO 34 J=1,K
      S=AK(7,J)*L(J)**2
   34 SS=SS+S
      CA=2.*(BETA2/H**2+SS)
      CB=1.0+SIG+BETA1/H
      NY=0
   33 YM=M
      FX=CA*YM**2+CB*YM-TM
      FXP=2.*CA*YM+CB
```

ERRATA

Page	Line No.	
19	28	"... alcoholic ..."
20	4	"... binding sites from ..."
22	29	"[LH] = total COOH - ..."
23	3	"total COOH = ..."
44	9	" $-\log \gamma$ = ..."
47	equation 2.38	$10^{-pH} = \frac{K_a(v_e-v)C/(V+v)}{vC/(V+v)}$
55	11	"... in Milli-Q ..."
63	21	delete bracket,)
67	Figure 4.1a	"thermostated water"
72	17	i.e. weight of fulvic acid/(moles of titratable acid groups)
93	23	"... $\pm$ 0.16 $(Al(LH_{-1})_2^{5-}$."
121	10	"6.2 THE EXTRACTION METHOD"
134, 135		x-axes should read $[A]^{-1}$ y-axes should read T_A^{-1}
143	3	"... are also recovered ..."
150	22	"... greater than 100°C ..."
153	16	"... 3000-3600 cm^{-1} ..."
175	5	"... of monoprotic acids ..."
176	17	"... are calculated for each ..."
189	25	"... (9.0×10^{-8} M) ..."
202	18	"... models (iii), (iv) and (v) ..."
209	21	"Carboxyl gorups of substituted ..."
212	18	"... extent; ..."
229	14	"... using differential pulse ASV."
233	10	"For these complexes ..."
250	18	delete "lead"
250	28	"... in the presence of fulvic ..."
253	24,32	
254	32,34	"Powell, H.K.J."
255	12,29,31,34	
257	7,9	